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CONTENTS

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Contents

CORRIGENDA IN VOL. 40

p. 174.   *For* Fig. 2 *read* Fig. 3.
p. 175.   *For* Fig. 3 *read* Fig. 2.

GENETIC STUDIES IN POULTRY

XI. THE LEGBAR

BY R. C. PUNNETT

(With Plate 1 and Two Text-figures)

IN a paper which appeared in this *Journal* in 1930 Mr M. S. Pease and I showed that the barring factor, **B**, produces a markedly different visible effect on the down of the Campine fowl according as it is present in a homozygous or heterozygous condition. In the homozygous bird the production of pigment in the down is far more strongly inhibited, with the result that **BB** chicks are markedly paler than **Bb** ones. Since **B** is sex-linked all male chicks are **BB** and all female chicks **Bb**, and the former can at once be distinguished from the latter by the difference in colour (cf. *J. Genet.* **22**, Pl. 17). Moreover we pointed out at the time that a stock of "pure" Cambar hens, for so we have named the breed, could be rapidly increased by mating the **BB** cock to Campine hens. For all hens so bred would be **Bb** on a Campine basis and would breed as Cambars.

More recently Fisher (1935) showed that the barring factor behaves similarly with regard to the brown striped down of *Gallus bankiva*, while Hagedoorn (1935, 1936) has published briefly in connexion with the formation of auto-sexing breeds founded on Barnevelders and Leghorns. The general principle underlying the formation of such breeds has been discussed in more detail by Pease (1936).

The present paper records some further experiments of a similar nature on the brown-striped down of the Brown Leghorn. For the introduction of the barred factor into the Leghorn use was made of the Plymouth Rock, and in 1935 a hen of this variety, imported from Canada, was mated with a Brown Leghorn cock. In 1936 one of the barred cocks from this mating was run with three Brown Leghorn ♀♀, reared from eggs imported from Denmark in the previous year. The Danish strain was made use of because in that country it is a favourite breed and has been brought to a high pitch of excellence both for hardiness and egg production. It turned out to be somewhat darker in colour than the usual run of Brown Leghorns in this country, and the down colour of the chicks

was also darker and sharper in the striping. From the mating between the F_1 ♂ and the Brown Leghorn ♀♀ three main colour classes appeared, viz. blacks, silver greys and browns. The actual numbers were 57 blacks,

Pl. Rock ♀ × Br. L. ♂   1935
|
Barred ♂ × 3 Br. L. ♀♀   1936
(Danish)

Browns	Silver Greys	Blacks
Barred　Unbarred	Barred　Unbarred	Barred　Unbarred

♂ × 3 ♀♀   1937

Het. ♂♂　Hom. ♂♂ × ♀♀
(Bb)　　**(BB)**　　**(Bb)**

Legbars
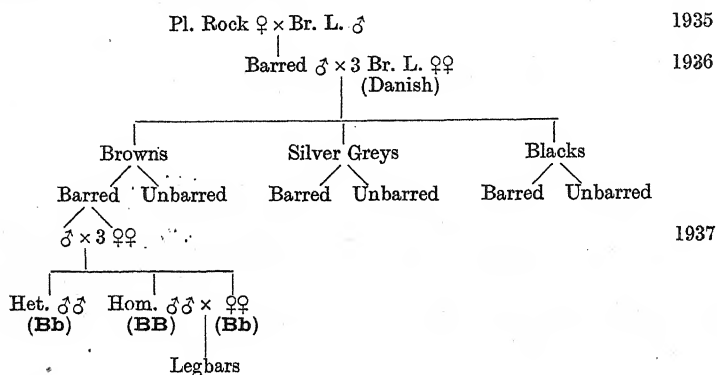
30 silver greys, and 40 browns where expectation is 2 : 1 : 1. Both barred and unbarred birds appeared in each class. From them were selected four barred birds of the brown class (viz. a cock and three hens) which approached most nearly to the Brown Leghorn type in colour and general habit; and in 1937 these were mated together in order to obtain the homozygous barred cocks. The majority of these were easily recognized in the down through their pale colour (cf. Pl. 1, fig. 2). They were also characterized by a marked light head patch and by a blurring of the light dorsal rump striping. Some homozygous **BB** ♂♂ were however in general depth of colour not far removed from a normal brown striped down (cf. Pl. 1, figs. 1 and 5). But further experience showed that the homozygous males could be distinguished from brown-stripes heterozygous for barring (cf. Pl. 1, fig. 4) by the marked light head patch, and especially by the blurring of the light striping on the rump. In **Bb** chicks of either sex there may be indications of a light head patch, but in this material it was never very marked, and might be absent. But in all **Bb** chicks the rump striping is sharply marked, whether the tone of the brown be very dark, as in the Danish strain, or lighter, as in most of the strains in this country (cf. Pl. 1, figs. 3 and 4).

Breeders of Brown Leghorns are aware that the tone of the down colour shows a considerable range of variation, probably due, as will appear later, to definite genetical factors. On the lighter type of Brown Leghorn down the barring factor in a homozygous condition produces its most marked effect, such as is shown in fig. 2 of Pl. 1. On the darker type its effect is to produce a down of which the general tone is not far

removed from that of the lighter type where the barring factor is in a heterozygous condition (cf. Pl. 1, figs. 1 and 5). Possible confusion is, however, eliminated when it is recognized that the darker **BB** type always shows blurring of the light rump stripes as well as a light head patch, whereas in the lighter **Bb** the head patch is small or absent and the light rump stripes are sharply defined. Although the Legbar, the name we give to this new breed, exhibits, like the Brown Leghorn, some variation in the intensity of pigment in the down of the chicks, there is nevertheless a perfectly clear distinction between the **BB** male chicks and the **Bb** females.

THE ADULT PLUMAGE

As might be expected from a breed founded on the Brown Leghorn, the Legbar shows a marked sexual dimorphism in the plumage. The hen, as shown in Text-fig. 1, is not unlike a Brown Leghorn in general
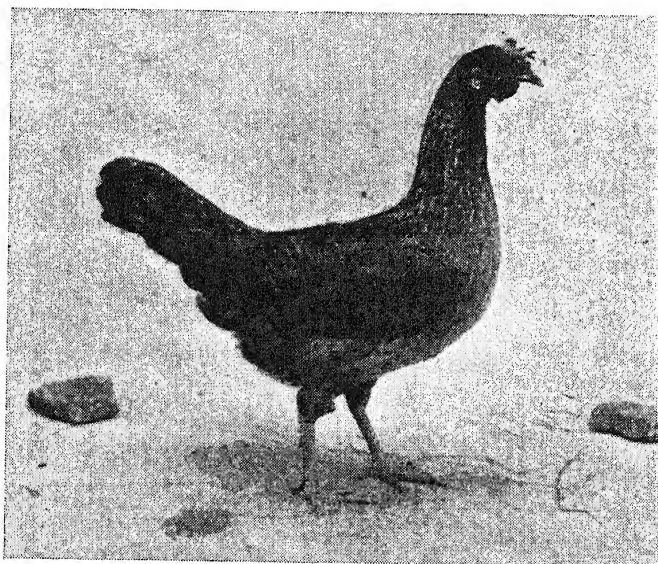
Text-fig. 1. Legbar pullet.

appearance, though the gold of the hackle and the salmon of the breast are rather less intense. It has, however, a dingier look owing to the blurred barring in the body feathers and tail. The cock on the other hand is a strikingly handsome bird. As Text-fig. 2 shows, he is barred all over, though the general effect is definitely lighter and softer than in a barred

breed such as the Plymouth Rock. At the same time the pale gold of the hackles and the bright chestnut of the wing coverts lead to his presenting an appearance at once brilliant and quite unlike that of the male of any recognized breed.
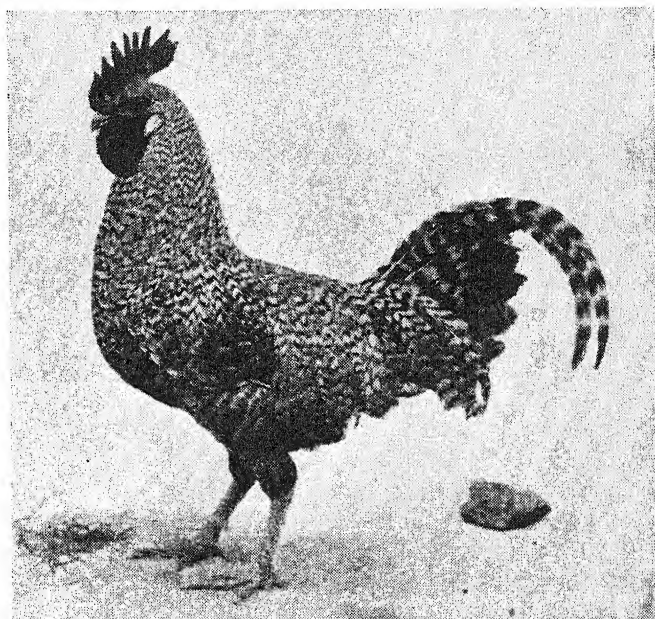
Text-fig. 2. Legbar cockerel.

THE RELATION BETWEEN DARKER AND LIGHTER DOWNS

In 1939 some further experiments were made with a view to discovering the relation between the darker and lighter types of down that had been encountered, and the following four pens were mated up.

Pen 11, ♂ 184/38 × ♀ 441/38, both of which had downs of the darker type. The resulting twenty-two chicks were classified as follows:

		Darker	Lighter
♂♂		8	1
♀♀		10	3
	Total	18	4

The proportions in which the two types appeared are consistent with the view that the darker type is dominant, and that the parents were both heterozygous.

Pen 12, ♂ 497/38 × ♀ 503/38. Here the ♂ approximated to the darker

type and was presumably heterozygous, while the ♀ was of the lighter type. The twenty-two chicks hatched were as follows:

	Darker	Lighter
♂♂	4	8
♀♀	5	5
Total	9	13

On the assumption that ♂ 497 was heterozygous, expectation for the two types is equality.

Pen 13, ♂ 496/38 × ♀ 112/38. Here again the ♂ approximated to the darker type while the ♀ was of the lighter type. The twelve chicks were distributed as follows:

	Darker	Lighter
♂♂	6	2
♀♀	3	1
Total	9	3

Pen 14, ♂ 451/38 × ♀ 164/38. The ♂ was here of the lighter type while the ♀ was a dark brown stripe though not quite so dark as some that were bred. The seventeen chicks were as follows:

	Darker	Lighter
♂♂	3	5
♀♀	3	6
Total	6	11

Expectation here again is equality.

Taking Pens 12, 13, and 14 together, where equality is expected the totals are 24 of the darker and 27 of the lighter type.

In 1940 a further test was made by mating ♂ 137/39 (a bird with the darker type of down derived from Pen 11/39) with three lighter type ♀♀ all from Pen 12/39. Of the twenty-six male chicks eleven were of the lighter and fifteen of the darker type; of the eighteen female chicks eight were of the lighter and ten of the darker type. Taken altogether the figures are consistent with the view that there is a definite factor leading to darkening of the down, which was doubtless brought in by the dark downed Danish strain, and that this factor reacts as a dominant to the ordinary lighter brown-striped type. Though in the great majority of cases there is no difficulty in referring a down, whether ♂ or ♀, to the darker or lighter class, there are occasional cases which give rise to hesitation, and I incline to think that there may be minor factors involved which push, as it were, the heterozygous bird in the one direction or the other. Nevertheless the general dominance of the darker over the lighter type is unmistakable.

SHANK COLOUR

When hatched the shanks of Legbar chicks show a definite slaty or willow tinge, and this at first seemed puzzling in a breed based upon the Brown Leghorn which contains the dominant **Id** factor for light shank. The point proved an interesting one, and light has been thrown upon it through some further experiments upon the influence of the barring factor on brown downs. In 1938 a mating was made between a Legbar cock and a Brown Sussex hen, a breed which is also brown-striped in the down. In 1939 two F_1 ♂♂ were each mated back to several Brown Sussex hens, producing of course barred and unbarred birds of both sexes. About 100 birds were reared and from the barred ones were selected two pens from those birds which most closely approached the Brown Sussex in general type. For one of the objects of the experiment was to produce an auto-sexing breed of table poultry. At the time these chicks were hatched the point in connexion with the tingeing of the shanks had not arisen, and no particular attention was paid to this feature as the chicks emerged. Incidentally, however, the tingeing of the shanks was recorded in a certain number of cases. When it was noticed, on going over the records at the end of the season, that all such cases were confined to the female sex it was realized that a point of interest had arisen, and the shank colour on hatching was carefully noted in 1940, in which year two pens were mated up from among the barred birds bred in 1939. It should be stated that this phenomenon of tinged shanks is a transient one, and in no case did a bird which hatched with tinged shanks fail to develop a light shank colour, whether white or yellow, on approaching maturity.

Before considering the 1940 data we may briefly outline what appears to be the explanation of this phenomenon of shank tingeing. In the Legbar × Brown Sussex cross we are dealing with two factors inhibiting mesodermal shank pigmentation, viz. the shank inhibitor, **Id**, and the barring factor, **B**. As I pointed out in a recent paper (1940), these two factors act upon the shank pigmentation in a different manner. **Id** chicks, from hatching onwards, always show a light shank except for an occasional "shot-mark", i.e. the inhibitory action of **Id** on the meso-dermal pigmentation occurs at a relatively early stage. On the other hand, **B** does not completely suppress the development of the mesodermal shank pigment until a later stage. **B** chicks, in the absence of **Id**, show some mesodermal pigmentation in the earlier stages though the barring factor gradually leads to its suppression before the bird reaches maturity.

For no adult barred bird is ever dark shanked. Further, **Id** and **B** are closely linked in the sex-chromosome (cf. Punnett, 1940). On this view we may now examine the data gathered in 1940. And it will perhaps help to clarify the argument if I give a brief scheme of the Brown Sussex × Legbar crosses accompanied by what I consider to be the genetical interpretation involved. For the sake of simplicity I have in this scheme assumed that **Id** and **B** are so closely linked that cross-overs may be neglected. Actually, as will appear later, a few cross-overs do occur.

<pre>
        Brown Sussex ♀ × Legbar ♂   (1938)
           Idb.idb   │ idB.idB

              F₁ ♂   × Brown Sussex ♀   (1939)
            Idb.idB   │   Idb.idb

               Barred ♂ × Barred ♀   (1940)
               Idb.idB │ idB.idb

   ♂♂   ♂♂   ♀♀   ♀♀
Homozygous barred Heterozygous barred Barred   Unbarred
   idB.idB   idB.Idb   idB.idb   Idb.idb
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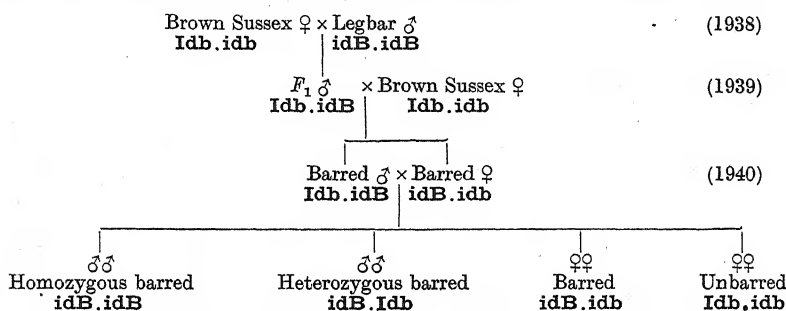
The 1940 mating between barred male and barred female should on this scheme give rise to four classes of birds, viz. homozygous and heterozygous barred ♂♂, barred (heterozygous) ♀♀ and unbarred ♀♀, and these four classes should be produced in approximately equal numbers. Such was the case. But in so far as shank tingeing is concerned these four classes should show definite distinctions. For since **BB** ♂♂ lack **Id** they should show tinged shanks, while the **Bb** ♂♂ should hatch with light shanks.[1] Again, the barred ♀♀, lacking **Id**, should hatch with tinged shanks, and the unbarred ♀♀, containing **Id**, should hatch with light shanks. The actual data obtained are as follows:

		BB ♂♂	**Bb** ♂♂	♀♀
Light shank:	barred	—	18	3*
	unbarred	—	—	16
Tinged shank:	barred	13	1*	18
	unbarred	—	—	—

* Cross-overs.

These data are in full accordance with the explanation offered on the assumption that about 6% of cross-overs occur. Elsewhere (Punnett, 1940) I have argued for a cross-over value of about 10% between **Id** and **B**. The data are at present too scanty to fix this value with any precision.

A few further data may be given in support of the hypothesis ad-

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[1] The distinction between **BB** and **Bb** ♂♂ is similar to that described for the Legbars, so that these two classes can be distinguished in the down.

vanced. In 1940 another barred ♂, constitutionally **Idb. idB** was mated with several Brown Sussex hens (**Idb. idb**). Apart from rare cross-overs expectation is here equal numbers of barred and unbarred ♂♂ all with light shanks, together with equal numbers of unbarred ♀♀ with light shanks and barred ♀♀ with tinged shanks. The actual figures were as follows:

		♂♂	♀♀
Light shank:	barred	10	1*
	unbarred	12	5
Tinged shank:	barred	—	9
	unbarred	—	—

* Cross-over.

Here again the experimental data are evidently in close accord with the hypothesis outlined.

These experiments enable us to see how it is that the Legbar hatches with tinged shanks. For in selecting for barring we have at the same time selected against the inhibitory shank pigmentation factor owing to the "repulsion" that exists between **B** and **Id**. We have unconsciously produced a breed in which the **Id** factor of the Leghorn has been eliminated through selecting for barring. The Legbar is really a breed with mesodermal shank pigment derived from the Plymouth Rock, but, as in all barred breeds, the expression of the pigmentation factor is inhibited by that for barring as the bird grows to maturity.

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EXPLANATION OF PLATE 1

Fig. 1. Darker ♂ (**BB**) type.   Fig. 3. Darker ♀ (**Bb**) type.

Fig. 2. Lighter ♂ (**BB**) type.   Fig. 4. Lighter ♀ (**Bb**) type.

Fig. 5. Darker ♂ (**BB**) type.
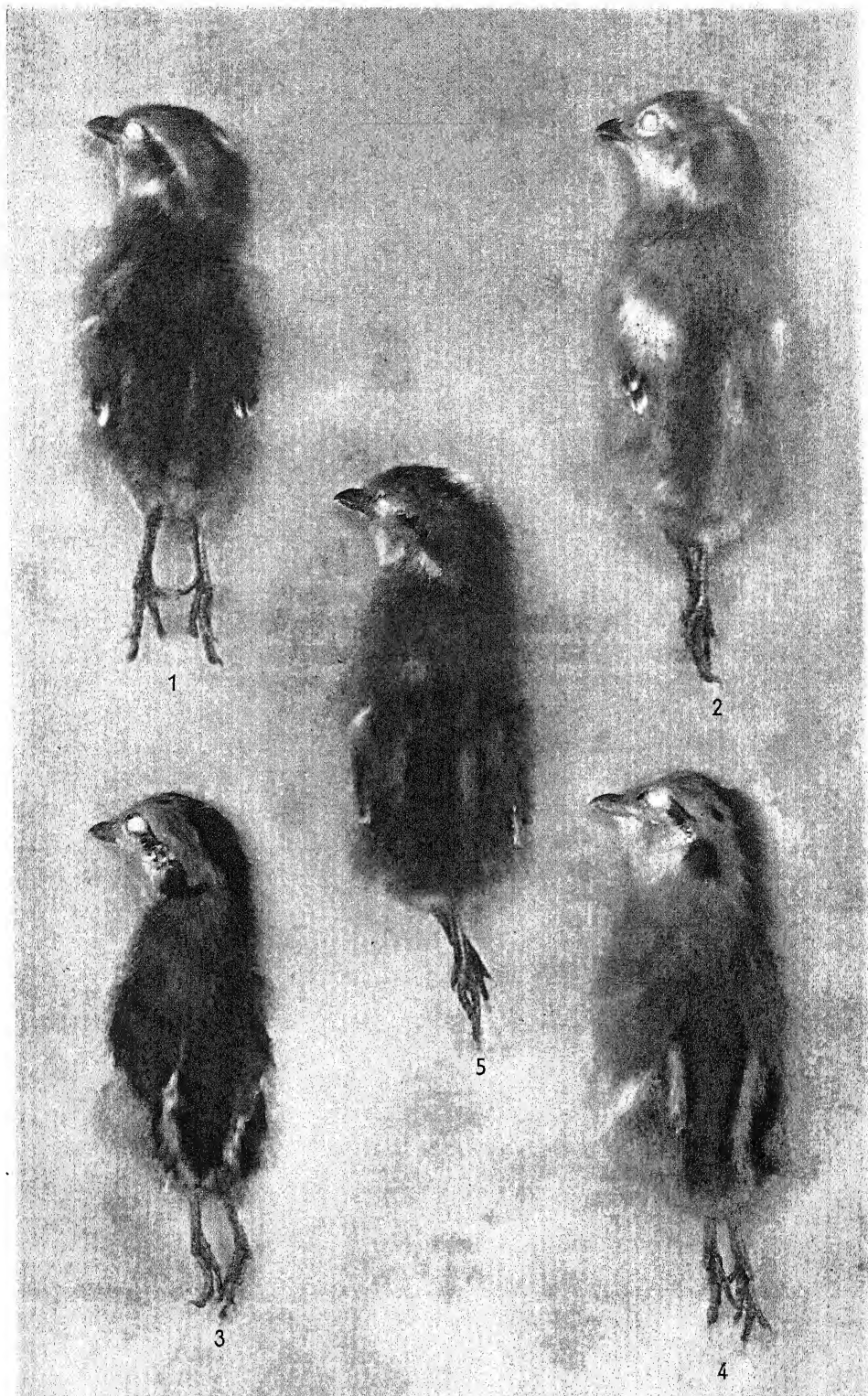
THE CAUSAL SEQUENCE OF MEIOSIS

I. CHIASMA FORMATION AND THE ORDER OF PAIRING IN *FRITILLARIA*

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(With Twenty-seven Text-figures)

1. INTRODUCTION

IN a recent survey of our present knowledge of pairing and chiasma formation in meiosis, Darlington (1940 b) distinguishes three "prime variables of meiosis" which "affect where pairing begins and how far it proceeds", and where crossing-over takes place. These prime variables are the contact-point, the time-limit of pairing and the torsion in paired regions. Their action has been inferred from the comparison of pairing in related forms with variable pairing frequencies and chiasma distribution; from the pairing in hybrids, mutants and polyploids; and from the artificial interruption of normal pairing. Such comparisons have to be made with even greater accuracy and detail if the theory of meiotic pairing is to be tested and consolidated. The time has now arrived for an exact definition of the individual actions and of the interactions of the three variables and of their effect on chiasma formation.

A study of pairing variation resulting from genetic and from environmental sources, i.e. an interspecific comparison with varied environmental conditions, forms the most promising approach. *Fritillaria*, which has proved such useful material in the discovery of the elements of mitosis and meiosis, is eminently suitable for further analysis, for the following reasons:

(1) It has chromosomes of exceptional length.

(2) It has some chromosomes with subterminal (S) and others with submedian (M) centromeres.

(3) Chiasmata retain their diplotene positions almost unchanged until metaphase. They do not merge and their only change in position is a slight distal movement of the chiasmata furthest removed from the centromere (Darlington, 1936). These observations are in agreement with Mather's (1939) recent conclusions on chiasma movements of non-terminalized bivalents.

(4) It includes species with chiasma frequencies from 1·7 to 5·0 chiasmata per bivalent.

(5) It includes species with varying degrees of localization.

After a detailed study of one species with localized pairing (Newton & Darlington, 1930) and one with non-localized pairing (Darlington, 1930), a survey of twenty-five species showed that every degree of localization existed, from the extreme proximal localization of *F. ruthenica* to the unrestricted, complete pairing of *F. imperialis* (Darlington, 1936). Differential prophase pairing explains these differences in chiasma distribution (Darlington, 1935). Pachytene chromosomes of all species show differential condensation of the proximal parts which goes with their precocity of pairing; the *contact-point* lies in the centric region. Thence pairing proceeds in a distal direction until interrupted by the *time-limit*. This may be early, when pairing is restricted to the proximal region; it may be late, when pairing is complete; or it may be intermediate, when pairing is partial, or intermittent. *M* bivalents which are hindered in their movement by having their centromeres tethered to long arms on either side of them, have a later pairing start and therefore restricted pairing in relation to *S* bivalents. All pairing is open to variation, especially in species with intermediate localization. Thus bivalents with extreme proximal localization are found in *F. imperialis* or bivalents with almost complete pairing in *F. meleagris*. Thus localization—"procentric" or "proterminal" (Darlington, 1940*b*)—is no absolute category rigidly divided from "free pairing", but merely one end of a graduated scale, with exceptional bivalents emphasizing the natural relationship. Finally, Darlington (1936) has inferred different degrees of *torsion* from the differences in chiasma frequency between species with equal localization and between clones of *F. imperialis* with free pairing.

This paper is an attempt to utilize a statistical survey of chiasma distribution in a number of species of *Fritillaria* for a further test of the action of the prime variables of meiosis. Results thus obtained may serve as a starting point for a study of pairing differences induced by environmental variation.

2. Material and methods

For this study a number of species were selected which vary in localization and chiasma frequency. Dr Darlington's collection of *Fritillaria* preparations was kindly made available. All preparations were made by Mr L. La Cour (for methods of fixations, cf. Darlington, 1936).

The following is a list of species used, together with the number of nuclei studied:

Species	Abbreviation	Number of nuclei	
F. acmopetala	*A*	15	
F. pontica	*Po*	15	
F. latifolia	*L*	10	
F. meleagris	*M*	15	
F. meleagroides	*Md*	10	Including one nucleus from
F. hispanica	*H*	10	Darlington (1936, p. 280)
F. pallidiflora	*Pa*	5	
F. imperialis	*I*	5	
F. ruthenica	*R*	15	From Darlington (1936, p. 277)

The clone of *F. imperialis* used had a chiasma frequency of 4·30 per bivalent, i.e. it is one of those with highest chiasma frequency.

The only reliable distinction among metaphase bivalents in *Fritillaria* is that between median or submedian (*M*) and subterminal (*S*) chromosomes. Within these classes there are marked differences in the length of arms and of chromosomes, insufficient however for regular identification of individual chromosomes. All species studied possess in their haploid set ten *S* and two *M* chromosomes. Only *F. ruthenica*, for which some of Darlington's results (1936) were used, has four *S* and five *M* chromosomes.

The range of chromosome lengths in one species, *F. latifolia* var. *major*, and *F. imperialis* is indicated by the following measurements in μ (Darlington, 1940*a*):

Arms:	*F. latifolia*		*F. imperialis*	
	Longer	Shorter	Longer	Shorter
Longest *S* chromosome	20·0	2·0	14	1
Shortest *S* chromosome	14·0	1·2	10·5	0·5
M_1	17·2	16·0	11	9
M_2	12·0	7·2	12	7
Proportion *S* : *M*	0·7 : 1		0·7 : 1	

Thus, in both species of the four *M* chromosome arms three are within, or close to, the range of the long arms of the *S* chromosomes, but the fourth is only half as long as the long arm of the shortest *S* chromosome.

The method of approach was a quantitative study of the regional distribution of chiasmata in all bivalents of random cells. Measurements of distances between metaphase chiasmata can be reliably corrected only where individual chromosomes can be identified. Chiasmata were therefore classified by an estimation of their position in relation to the centromere and to the distal ends. The short arm of *S* bivalents was

treated as one pairing unit, the long arm of S bivalents and both arms of M bivalents were subdivided into three "regions":

	S bivalents	M bivalents
C Centric region	SA Short arm	
	P Proximal region	P Proximal region
	M Median region } Long arm	M Median region
	D Distal region	D Distal region

For record purposes diagrams were drawn of all bivalents studied (cf. Figs. 1, 13). Some of the bivalent types are illustrated in Figs. 3–12 and 15–19.

With the exception of the SA region, the delimitation of pairing regions is arbitrary and, especially in bivalents with a high chiasma frequency, it is open to a certain degree of error. It is however consistent. The single chiasma in the long arm in 1a and s1a (Fig. 1) is "proximal",

Fig. 1. Types of S bivalents in *Fritillaria* which have been, or could be seen. Upper half, bivalents with short-arm pairing; lower half, bivalents without short-arm pairing.

in 1b and s1b "median", in 1c and s1c "distal". Similarly, where the proximal loop is small, the first and second chiasmata are classed as proximal (e.g. in 2a, 3a, 3c, 4a, 4b, s2a, s3a, s3c, s4a, s4b, etc.). Where the proximal loop is larger, as in 2b, 3b, 4c, s2b, s3b and s4c, the first chiasma is called proximal, the second median, and pari passu for further chiasmata. In order to separate the median and distal regions with sufficient consistency, only terminal or nearly terminal chiasmata were

scored as distal; hence, whilst two chiasmata are common in each of the other two regions, they were scored in the distal region only where the distal loop was exceptionally small (2e, 3e, 6, s2e, s3e, s6). Thus the distal region occupied a smaller chromosome sector than the proximal and median regions, a fact which should be taken into account when regional chiasma frequencies are compared.

In the short arm only one single chiasma has been recorded wherever chiasma formation occurred. Whilst two chiasmata have been seen occasionally, their appearance is too rare to affect chiasma frequencies, and since the pairing region is very short, an extra chiasma does not affect pairing frequencies.

In general, "pairing" is applied as synonymous with "chiasma formation". The term "pairing frequency" however represents the frequency of association, irrespective of the number of chiasmata.

3. CHIASMA FORMATION IN S BIVALENTS
Chiasma frequencies

The chiasma frequencies of the eight species are listed in Table I. They rise from 1·97—one of the lowest frequencies in S bivalents of *Fritillaria*—to 4·24, which is one of the highest. Darlington (1936) recorded *F. acmopetala* with 3·00 chiasmata per bivalent and 2·97 chiasmata per S bivalent, whilst in the present study the corresponding data are 1·92 and 1·97. Presumably two different clones had been used.

TABLE I
Chiasma frequencies in eight species of Fritillaria

	A	Po	L	M	Md	H	Pa	I
Chiasma frequency per cell	23·0	27·4	27·7	29·0	32·8	34·5	46·8	51·6
Chiasma frequency per bivalent	1·92	2·28	2·30	2·42	2·73	2·87	3·90	4·30
Chiasma frequency per S bivalent	1·97	2·39	2·33	2·47	2·84	2·89	3·96	4·24
Chiasma frequency per M bivalent	1·65	1·75	2·20	2·20	2·25	2·80	3·60	4·60

Failure of chiasma formation

Failure of pairing is exceedingly rare. In all the nuclei studied and in the hundreds of cells cursorily inspected only one pair of univalents was found, viz. in a cell of *F. pontica*.

Regional distribution of chiasmata (Figs. 1, 2, 20, 22, 23 and Table II)

Using the method of classification described in the previous section, the types of metaphase bivalents which have been—or could have

been—observed in *Fritillaria* are set out in Fig. 1. Their frequencies are set out in Fig. 2.

Localization. Bivalents with a single *distal* chiasma (1c) do not occur; two distal chiasmata occur sporadically in *F. imperialis*. Bivalents with a single *median* chiasma (1b) are exceedingly rare and have been seen only in *F. pontica* (Figs. 7, 8). Two median chiasmata (2d) have not been seen. Bivalents with chiasmata confined to the *centric* region, i.e. the short arm and (or) the proximal region of the long arm, occur in all

Xta in S Bivalents (per 100)

Species (Xta per S biv)	Short Arm Xta	0	1a	1b	1c	2a	2b	2c	2d	2e	3a	3b	3c	3d	3e	4a	4b	4c	5a	5b	6
F. acmopetala 1·97			12			5															
	S 3		68			12															
F. pontica 2·39			10	2		3	1	1								1·					
	S 4		30			26	8	6				2	3	1		1					
F. latifolia 2·33			9			10	2					1	1								
	S 2		40			25	2	1			6	1·									
F. meleagris 2·46			7			13	1·5	1			1		1	1							
	S		33			25	6	1			5	1	2				1·5				
F. meleagroides 2·83						1															
	S 1		27			52	5	1			8		5								
F. hispanica 2·89			5			7	6	3			9		2	2		1	4	1	1	3	1
	S 5		12	1	1	13	7		1		6		3·			2	2	1		1	
F. pallidiflora 3·96						2	2				2	8	6	2		10	2		14		
	S		2			2	8	4			2	2	12			10	4		6		
F. imperialis 4·24										4·		6	2	2		6				2	4
	S				2	2	4		2	4			16	14		18			10	2	

Fig. 2. Frequency distribution of *S* bivalent types in *Fritillaria* (cf. Fig. 1).

species. Their frequencies are, roughly, inversely correlated with the total chiasma frequencies of *S* bivalents (cf. Table II). The frequency curves of proximally localized bivalents (Fig. 23) show that the eight species are grouped in four classes. Bivalents of *A* are invariably centrically localized. *L*, *M*, *Md* and *Po* are strongly localized, with from six to ten localized bivalents in one cell; *L* has the highest, *Po* the lowest degree of localization in this group. *H*, with a broad curve in the central region, is intermediate. In *Pa* and *I* no more than one localized bivalent occurs—very infrequently in *I*.

Chiasma distribution. When pairing frequencies in all pairing regions

are considered (Table II and Fig. 22), it is found that the eight species fall into four groups according to the frequency and proportion of median

TABLE II

Pairing and chiasma frequencies in S bivalents

Species	Failure	Locali-zation C	Inter-mittent $P+D$	Partial		Com-plete $P+M+D$	Summary			Chiasma frequency
				$P+M$	$D+M$		C	M	D	
F. acmopetala	0	100	0	0	0	0	100	0	0	1·97
F. pontica	1	75*	10	11	0	3	97	16	13	2·39
F. latifolia	0	86	3	11	0	0	100	11	3	2·33
F. meleagris	0	78	5	14·5	0	2·5	100	17	7·5	2·46
F. meleagroides	0	81	6	13	0	0	100	13	6	2·83
F. hispanica	0	42	10†	33‡	0	15	100	48	25	2·89
F. pallidiflora	0	4	18	12	2	64	98	78	84	3·96
F. imperialis	0	2	24	8	6	58	94	72	88	4·24

    * Two bivalents with M pairing.
    † One bivalent with $SA+D$ pairing.
    ‡ One bivalent with $SA+M$ pairing.

and distal pairing. As is to be expected, this grouping corresponds to that resulting from the frequency of localized bivalents.

(1) No median or distal pairing: A (Fig. 3). Chiasma formation is almost entirely confined to the centric region. In forty nuclei, twenty
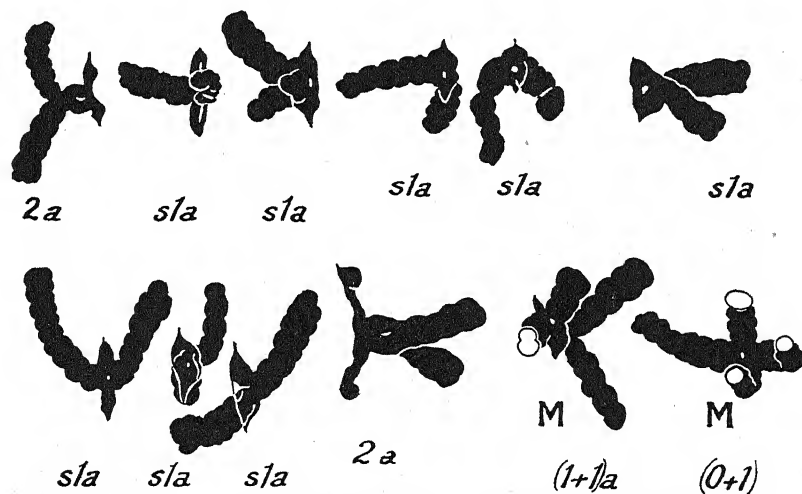

Fig. 3. Side-view of first metaphase in *F. acmopetala*. ×1900.

seen in side-view and twenty in polar view, only one exceptional S bivalent was found with one chiasma in the short arm and a distal chiasma in the long arm (Fig. 4).

(2) Median and distal pairing frequencies below 20%: *L, M, Md, Po* (Fig. 5). The great majority of bivalents pair in the proximal or in the proximal and median regions. Intermittent pairing $(P + D)$, with failure

Fig. 4. Exceptional bivalents in *F. acmopetala*; left, *S* bivalent 2c; right, *M* bivalent $(1 + 3)$ *c.* ×1900.
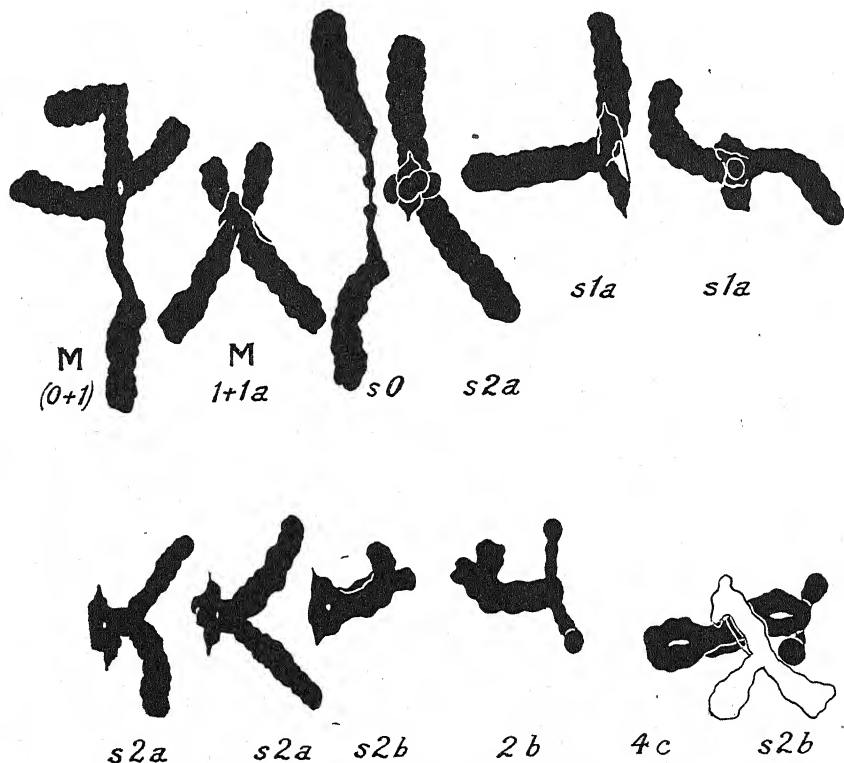
Fig. 5. Side-view of first metaphase in *F. pontica.* ×1900.

of pairing in the median region, occurs sporadically in all species (cf. Newton & Darlington, 1930, Fig. 7). Complete pairing is much rarer and is confined to *Po* and *M*. Thus distal pairing as a rule is not a *sequel* to median pairing, but independent of it. Complete pairing occurs not through an extension of proximal pairing through the median into the distal region, but by coincidence of partial $(P + M)$ and distal pairing.

There is then a primary, proximal, and a secondary, distal, contact-point. The first acts regularly, the incidence of the second is subject to the action of the time limit.

F. pontica differs from the other members of this group in three respects. Exceptional bivalents with median localization occur; there is rare failure of pairing, unknown in other species; the proportion of distal pairing is twice as high as that of any other species in this group. These observations can be explained on the assumption that in comparison with these species the time-limit of pairing in Po is later and that
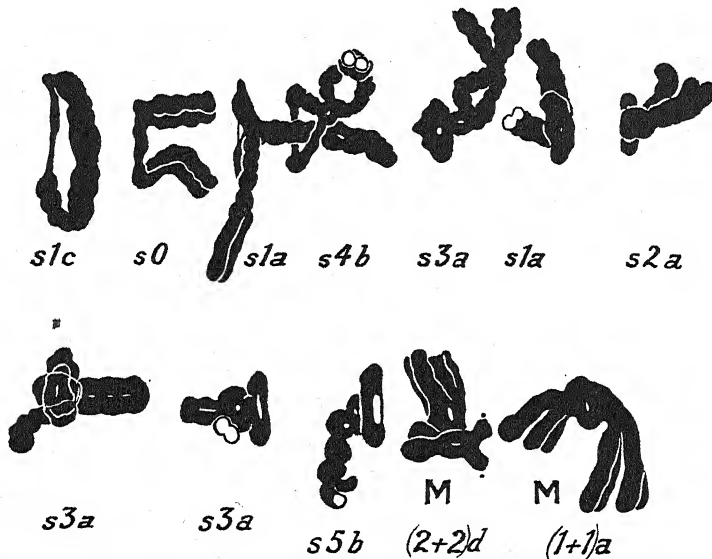
Fig. 6. Side-view of first metaphase in *F. hispanica*. All S bivalents have short-arm pairing (cf. Darlington, 1936, Fig. 5) (seven bivalents without short-arm pairing). × 1900.

consequently distal pairing is increased and centric localization reduced. *F. pontica* possesses neither the full co-ordination of the pairing mechanism of the highly procentrically localized species, nor the less precise, but no less effective co-ordination of the species with a late time-limit and a high frequency of pairing at both, centric and distal, contact-points.

(3) Median pairing frequency about 50, distal about 25 %: H (Fig. 6). In comparison with the second group, intermittent pairing is increased, with the rare types $s\,2d$ and $s\,1c$ (Figs. 9, 10) indicating a strong terminal attraction. Distal pairing is increased to 25 %. Yet pairing in the median region is due to an extension of centric pairing ($C \to M$) rather than of distal pairing ($D \to M$). This is shown in two ways: first, there are 33 % partially paired bivalents with $C + M$ pairing, but none with $D + M$

pairing, and, secondly, the observed proportion of complete pairing (15 %) is only slightly in excess of that calculated on the basis of 48 % $C+M$ pairing and 25 % independent D pairing, viz. 12 %.

(4) Median pairing about 75 %, distal pairing higher: *Pa, I*. The frequencies of distal pairing are only slightly below those of proximal pairing. Centrically localized bivalents (Fig. 11) are sporadic. Intermittent pairing is more frequent than in the previous group, and partial pairing $D+M$ occurs. The majority of bivalents are completely paired.

The most significant difference between this and the previous groups concerns the relation between the pairing in the median and distal regions. In the second, and especially in the third group, median exceeds distal pairing; in the fourth group the relation is reversed. This confirms
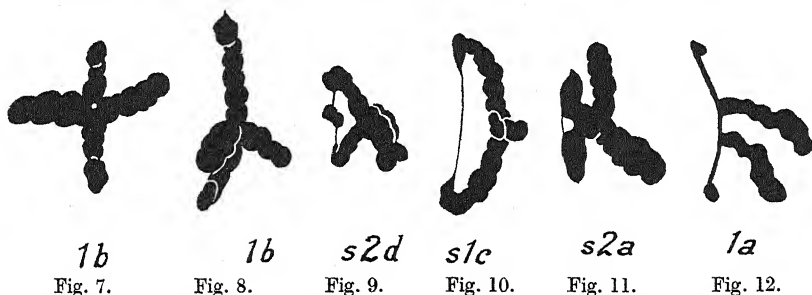

		s2d	s1c	s2a	1a
1b	1b				
Fig. 7.	Fig. 8.	Fig. 9.	Fig. 10.	Fig. 11.	Fig. 12.

Figs. 7–12. Rare *S* bivalents. Figs. 7 and 8. *F. pontica*. Figs. 9 and 10. *F. hispanica*. Fig. 11. *F. imperialis*. Fig. 12. *F. latifolia*: chiasma formation in a proximal inversion. × 1900.

the assumption that pairing starts proximally and distally and extends into the median region. This relationship of M and D pairing in the fourth group does not appear however if chiasma frequencies instead of pairing frequencies are considered (Fig. 20). This is due to the higher chiasma frequency in the M region. This no doubt is largely due to the shorter length of the D region (cf. § 2). The lower pairing frequency in the M region cannot be due to interference in the shorter S chromosomes. The majority of nuclei in *Pa* and *I* form at least one median chiasma, and in those bivalents which fail to pair in this region, the distal loop is large enough to permit at least one further interstitial chiasma.

In this as in the previous groups there is almost invariably a centric contact-point, but significant exceptions with only a distal contact-point occur. The time-limit of pairing is now considerably extended, permitting a high degree of distal pairing. Chiasmata in the median region may now be due to extension of pairing from either direction and there is no evidence which is more frequent. The precision conferred by

high centric localization has been partly lost, but is supplemented by increased distal pairing which bridges the gap in the rare cases in which the former fails.

Note on inversions in Fritillaria. Inversions in five species of *Fritillaria* have been recorded by the author (Frankel, 1937); and in one by Bennett

Fig. 13. Types of *M* bivalents in *Fritillaria* which have been, or could be, seen.

(1938). Of these six species four have unrestricted and two have localized pairing. In the course of the present study inversions in two further species have been seen, viz. in *F. imperialis*, with non-localized pairing, and in *F. latifolia*, with highly localized pairing. It is obvious that crossing-over in inverted segments must be more frequent in species with non-localized pairing, hence the proportion of observed cases of inversions in localized and non-localized species is according to expectation. In

localized species chiasmata in inversions are to be expected either close to the centromere, giving a very short bridge and a long fragment, or in

Xta in M Bivalents (per 20)

Species	0+1	0+2	1+1			1+2					1+3			
			a	b	c	a	b	c	d	e	a	b	c	d
F. acmopetala	7		13											
F. pontica	5	1	13	1										
F. latifolia	2	2	11				2	1	1					
F. meleagris		·	16				4							
F. meleagroides	3		10				4	2						
F. hispanica	1		9				4					2		
F. pallidiflora					4	2				2	2	4	2	
F. imperialis														

Species	2 + 2						2 + 3						2+4	
	a	b	c	d	e	f	a	b	c	d	e	f	a	b
F. acmopetala														
F. pontica														
F. latifolia			1											
F. meleagris														
F. meleagroides	1													
F. hispanica	2			1				1						
F. pallidiflora										2	2			
F. imperialis					6	4					6	2	2	

Fig. 14. Frequency distribution of *M* bivalent types (cf. Fig. 13).

the distal region—resulting from rare distal pairing—which would give a long bridge and short fragment. The latter is the case in *F. citrina* (cf. Frankel, 1937, Figs. 32, 33) and in *F. ruthenica*. *F. latifolia*, the third

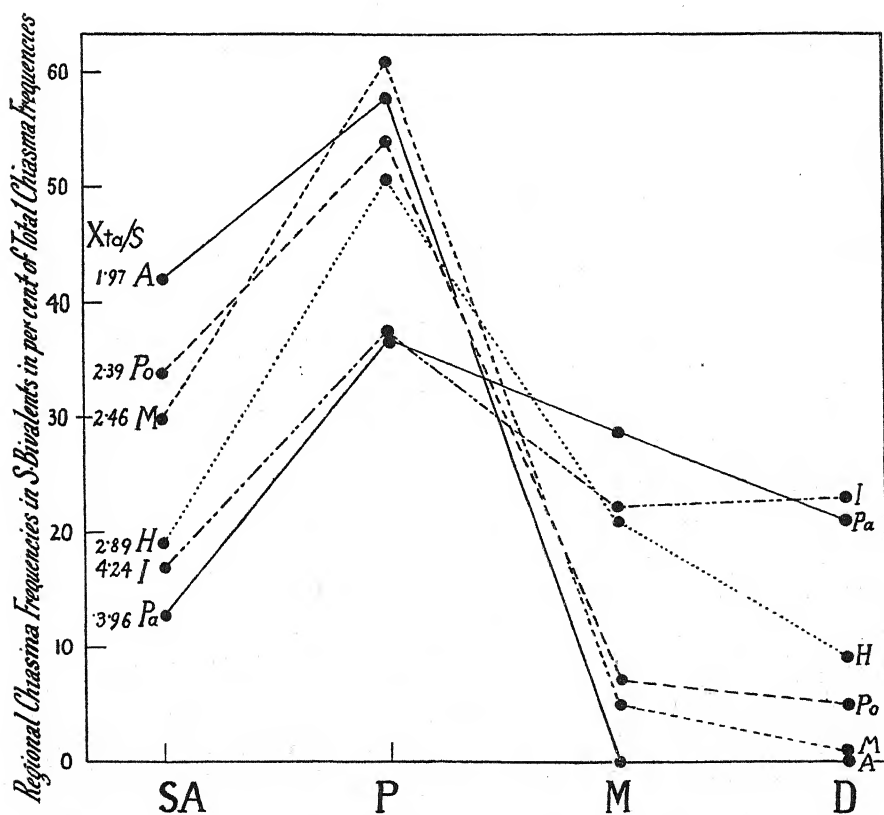(0+2)   (1+2)c   (1+1)b   (1+1)c   (1+1)c

Fig. 15.   Fig. 16.   Fig. 17.   Fig. 18.   Fig. 19.

Figs. 15–19. Rare *M* bivalents. Figs. 15 and 16. *F. pontica*. Fig. 17. *F. latifolia*. Figs. 18 and 19. *F. pallidiflora*. ×1900.

Fig. 20. Regional chiasma frequencies in *S* bivalents.

species, apparently has a proximal inverted segment (Fig. 12). The bridge resulting from this fragment is likely to break in early anaphase; the fragment is probably larger than any acentric fragment which has so far been illustrated.

4. Chiasma formation in M bivalents

Chiasma frequencies are listed in Table I. Failure of pairing in M-bivalents has not been seen.

Regional distribution of chiasmata (Figs. 13, 14, 21, 22 and Table III)

Types of M bivalents and their frequencies are recorded in Figs. 13 and 14.

TABLE III

*Pairing frequencies in single arms (per 40 arms) and chiasma
frequencies of whole M chromosomes*

| Species | Failure | Localization | | Intermittent | Partial | Complete | | Summary | | | Chiasma frequency |
		P	D	$P+D$	$P+M$	$P+M+D$	O	P	M	D	
F. acmopetala	7	33	0	0	0	0	7	33	0	0	1·66
F. pontica	6	32	1	0	1	0	6	32	0	1	1·80
F. latifolia	4	31	0	2	3	0	4	36	3	2	2·20
F. meleagris	0	40	0	0	0	0	0	40	0	0	2·20
F. meleagroides	3	35	0	0	2	0	3	37	2	0	2·25
F. hispanica	1	30	0	1	4	4	1	39	8	5	2·80
F. pallidiflora	0	12	10	6	2	10	0	30	12	26	3·60
F. imperialis	0	0	0	24	8*	8	0	38	16	34	4·60

* Two arms with $D+M$ pairing.

Localization. Bivalents with a single *distal* association in one arm and without pairing in the other do not occur. Distal pairing in both arms, without proximal pairing in either, occurs very rarely in *F. pallidiflora* (Figs. 18, 19). Distally associated arms occur in combination with various kinds of pairing in the other, viz. $(1+1)b$, $(1+2)d$, $(1+2)e$, and $(1+3)d$, in *F. pontica* and in *F. pallidiflora*. Arms with none but *median* association have not been seen; but no doubt they would be hard to distinguish. *Centrically* localized bivalents prevail in the species of groups 1 and 2 (cf. the previous section). They are rare or absent in those of group 4.

Chiasma distribution. In the species with procentric localization, median and distal pairing is greatly reduced in comparison with that in S bivalents. Groups (1) and (2) are therefore merged.

(1) and (2) Median and distal pairing sporadic or absent: A (Fig. 3), Po (Fig. 5), L, M, Md. The great majority of paired arms are proximally associated. Partial pairing $(P+M)$ is very rare, intermittent pairing

occurs sporadically in L, complete pairing is totally absent. In A one exceptional arm with complete pairing has been seen; the other arm is proximally localized (Fig. 4).

(3) Median pairing about 20 %, distal pairing about 10 %: H (Fig. 6). Proximal pairing prevails, but partial, intermittent and complete pairing occur. Median exceeds distal pairing.

(4) Median pairing about 30 %, distal pairing considerably higher: Pa, I. Distal pairing is nearly as frequent as proximal and twice as frequent as median pairing. In M bivalents the "dip" in the frequency of median association occurs also in chiasma frequency graphs (Fig. 21), not only in pairing frequency graphs (Fig. 22), owing to the lower chiasma frequencies in comparison with S bivalents.

5. A COMPARISON OF PAIRING AND CHIASMA FORMATION IN S AND M BIVALENTS

(1) Chiasma frequencies per bivalent are higher in S than in M bivalents of all species except $F.$ $imperialis$. Since the length relationship of S to M is about 0·7 : 1, the chiasma frequency per unit length is higher in S than in M bivalents of all species.

(2) The rise in chiasma frequency, from lowest to highest, is proportional in S and M bivalents. The S/M factor is represented by a linear graph (cf. Darlington, 1940b).

(3) Failure of pairing in M bivalent arms of procentrically localized species is more frequent than that in the long arms of S bivalents:

(4) Pairing in the distal and median regions is considerably less frequent in M than in S bivalents of procentrically localized species (groups 1 and 2).

(5) In species with a high degree of distal pairing (group 4) the "dip" in the pairing frequency of the median region is more marked in M than in S bivalents (Fig. 22).

	Failure of pairing in arms (%)							
	A	Po	L	M	Md	H	Pa	I
Long arms of S bivalents	3	5	2	0	1	5	0	0
Single arms of M bivalents	17·5	15	10	0	7·5	2·5	0	0

Conclusions. The principles of pairing in S and M bivalents are the same. The first contact-point is almost invariably near the centromere, with a second one, where the time-limit permits, in the distal region. All differences in the pairing processes of S and M can be consistently explained on the assumption that pairing of M chromosomes is delayed in relation to that of S chromosomes (Darlington, 1936). This explains

their lower chiasma frequency, their more frequent failure of pairing of
long arms, the more restricted pairing in median and distal regions of
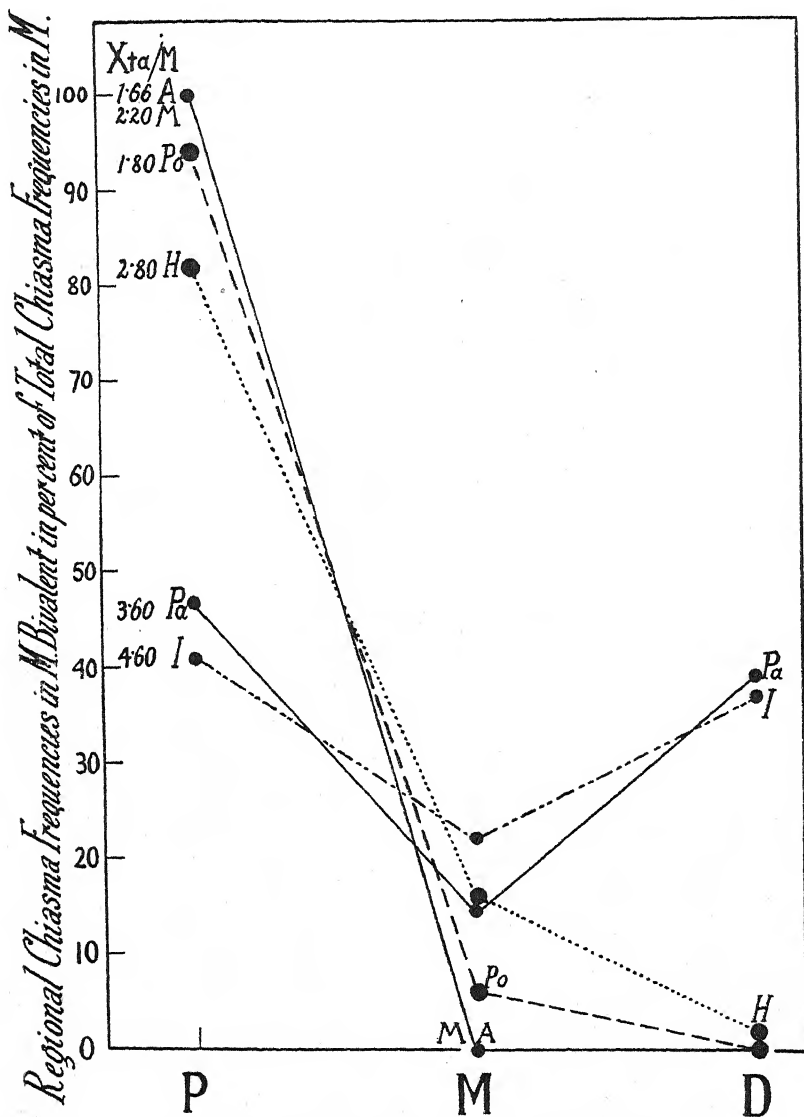

Fig. 21. Regional chiasma frequencies in *M* bivalents.

species with a short time-limit and, finally the lower frequency of com-
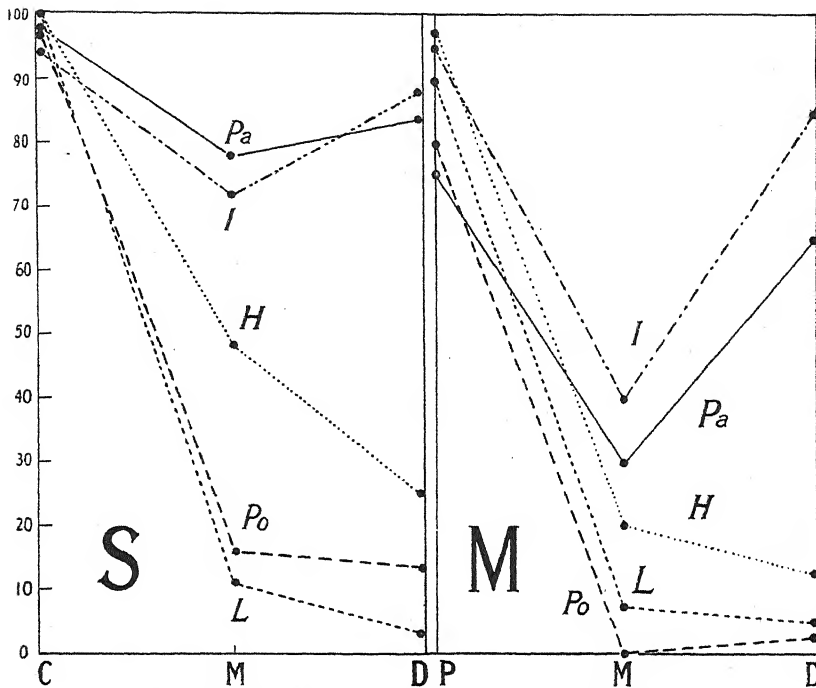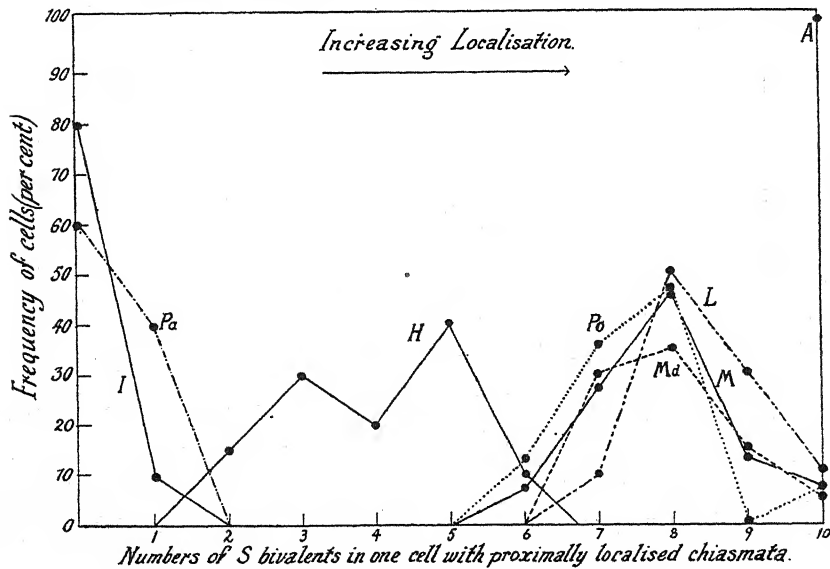plete pairing in species with a late time-limit. Length differences are

Fig. 22. Regional pairing frequencies in *S* and *M* bivalents.

Fig. 23. Frequency distribution of proximally localized bivalents.

partly responsible for differences in chiasma frequency, specially in the median region; but they do not suffice to explain the general trend of differential behaviour in the pairing processes of S and M bivalents.

6. THE RELATIONSHIP OF SHORT ARM PAIRING TO CHIASMA FREQUENCY IN S AND M BIVALENTS

(1) Using the chiasma frequency of M bivalents as a standard for interspecific comparisons, chiasma frequencies in the short and long arms of S bivalents exhibit a clearly defined relationship (Fig. 24). With a
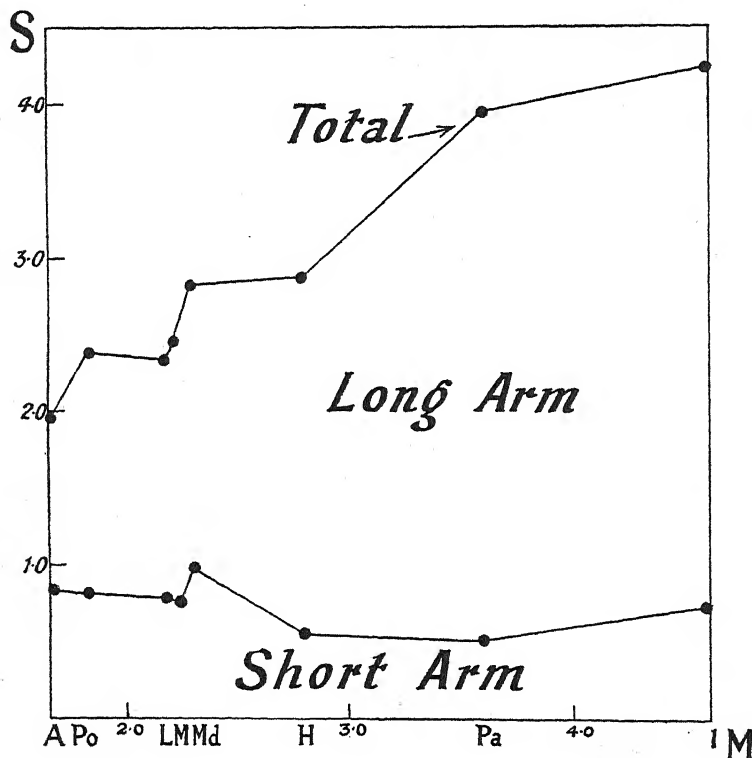
Fig. 24. Chiasma frequencies in the long and short arms of S bivalents in relation to chiasma frequencies in M bivalents.

rising chiasma frequency, an increasing proportion of chiasmata are formed in the long arm (LA) of S bivalents, a decreasing proportion in the short arm (SA). The absolute SA-chiasma frequency decreases with a rise in total chiasma frequency.

(2) The proportion of bivalents with SA chiasmata falls with increasing chiasma frequency (Fig. 25, upper part); but R, Md and I

exhibit *SA* pairing in excess of the expectation on the basis of a linear relationship.

(3) If however *SA* pairing is considered in relation to the total chiasma frequency in *S* bivalents, i.e. to the pairing behaviour of the organism of which the short arm forms a part, a linear relationship is established for all species except those with the lowest (*R*) and the highest (*I*) chiasma frequencies (Fig. 25, lower part). This shows that in general the chiasma frequencies of the two arms of *S* bivalents are balanced, i.e. that their variation is due to a single controlling system.
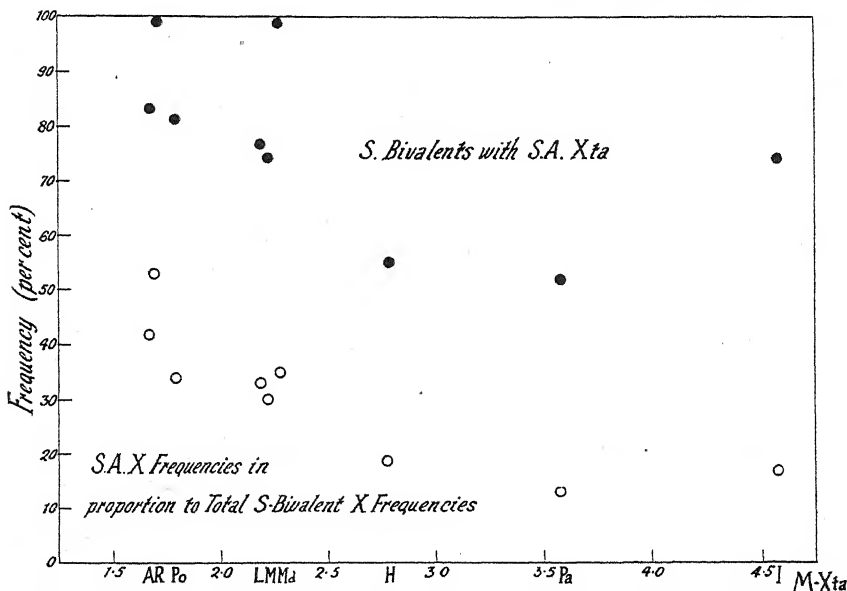
Fig. 25. Frequencies of *S* bivalents with *SA* chiasmata (black); *SA* chiasma frequencies in percentage of total chiasma frequencies (white circles); both in relation to chiasma frequency in *M* bivalents.

F. ruthenica is the only *Fritillaria* species known to have a chiasma frequency of the short arm greater than that of the long arm: This might be an effect of selection, if in this, the only species, with five *M* and four *S* chromosomes the *S* chromosomes with a shorter *SA* had "fused" and those with a longer short arm had been maintained. The latter, within the narrow limits of *SA* lengths, would have a greater chance of pairing. More probable however is an explanation on the basis of high torsion combined with an extremely early time-limit.

The species with the highest chiasma frequency, *F. imperialis*, also breaks the linear relationship. The longer time-limit, favouring terminal

association, might be thought responsible for the high chiasma frequency in the short arm, which possesses not merely centric but also terminal attraction. However a comparison of species with high and low *SA*-chiasma frequencies, but otherwise corresponding pairing behaviour, reveals that a difference in the intensity of *torsion* is more likely to account for the high chiasma frequency in the short arm of *F. imperialis* (Table IV). *M* and *Md*, *Pa* and *I* have similar chiasma frequencies in the median and distal regions, hence one may conclude that their time-

TABLE IV

Chiasma frequency in regions of *S* bivalents

Region	*M*	*Md*	*Pa*	*I*
SA	0·74	0·99	0·52	0·74
P	1·49	1·65	1·46	1·60
M	0·17	0·13	1·14	0·94
D	0·06	0·06	0·84	0·96

limits of pairing are similar; but *Md* and *I* exceed *M* and *Pa* in the degree of chiasma formation, i.e. in the *intensity of torsion*, in the centric region. The clone "No. 10" of *F. imperialis*, which has a higher chiasma frequency than that used in this study has, according to Darlington's figure (1930), a still higher degree of *SA* pairing.

(4) The resulting graph (Fig. 25, lower part) suggests a second degree curve, possibly a parabola. Within limits it permits a prediction of chiasma frequencies in *M* bivalents from the chiasma frequency percentage in the short arm of *S* bivalents of the same species.

(5) The question arises why *SA*-chiasma frequencies are higher in species with a short time-limit than in those with less restricted pairing. Interspecific differences in the intensity of torsion have been indicated above. It is suggested that in forms with restricted pairing the degree of torsion is increased by way of compensation, resulting in a higher chiasma frequency per paired unit length (see below).

(6) In any one species, the chiasma frequency of bivalents *with SA* chiasmata is higher than that of bivalents *without SA* chiasmata (Fig. 26, uppermost broken and full lines). This can be explained on the assumption that shorter arms start pairing more readily than longer arms and that consequently bivalents with *SA* pairing have an earlier start, an advantage in pairing time, over those with their contact-point in the proximal region of the clumsier long arm. If this assumption is correct, the rare distal chiasmata in procentrically localized species should occur in short rather than in long chromosomes. This has not yet been tested.
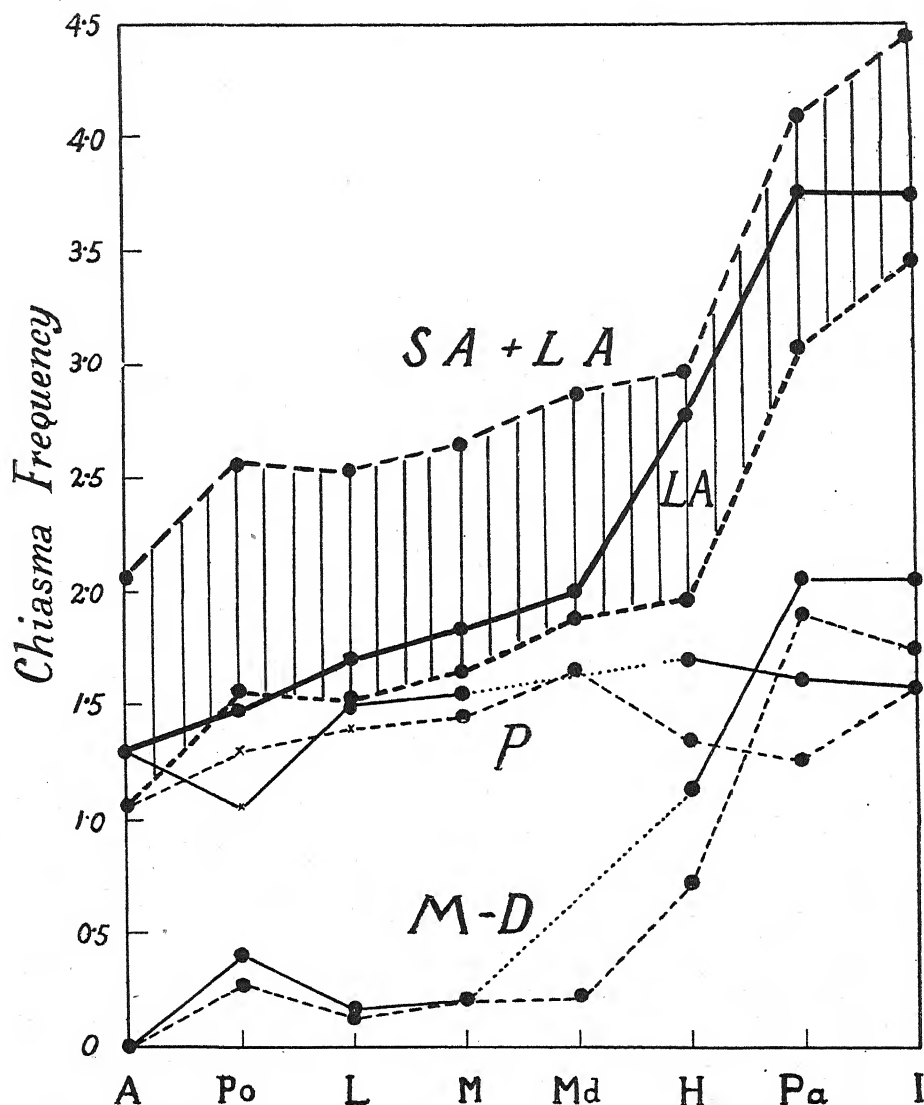
Fig. 26. Chiasma formation in *S* bivalents. Broken lines, bivalents with *SA* chiasmata; full lines, bivalents without *SA* chiasmata. (1) *SA + LA* chiasma frequencies in *S* bivalents with *SA* chiasmata. (2) *LA* chiasma frequencies in the long arms of bivalents without and with *SA* chiasmata. (3) *P* chiasma frequencies in the proximal region of the long arm. (4) *M–D* chiasma frequencies in the median and distal regions of the long arm.

(7) In all species except *F. pontica* the long arms of bivalents *without* SA chiasmata have a higher chiasma frequency than those of the bivalents *with* SA chiasmata (Fig. 26, first full and second broken line).

This difference in *LA*-chiasma frequencies may be due either to different lengths of the paired segments, or to different torsion in equal segments. If the former assumption is correct, bivalents without *SA* chiasmata should show an extension of the pairing region in a distal direction: the contact-point is shifted from the *SA* across the centromere into the *LA*, and the pairing segment is shifted correspondingly (Fig. 27).
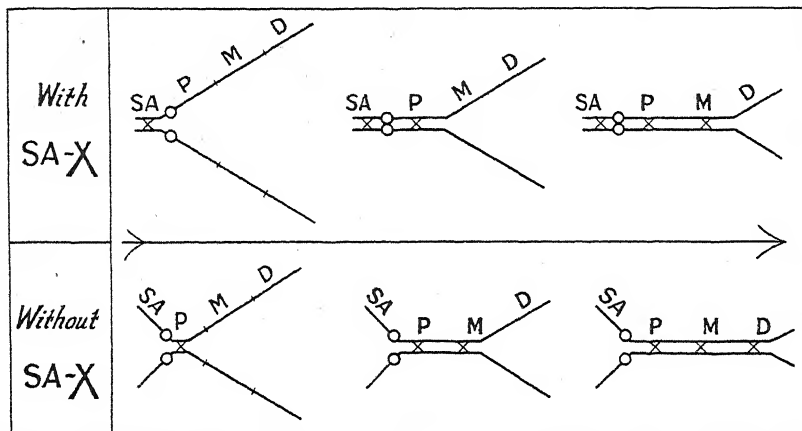
Fig. 27. The order of pairing in *S* bivalents with (above) and without (below) pairing in the short arm.

The methods of estimation and the number of bivalents used in this study do not permit a decision as to which of the two assumptions is correct. There is a slight but general tendency for increased chiasma frequencies in the proximal and (or) in the median-distal regions of *S* bivalents without *SA* chiasmata (Fig. 26, lower part). On general grounds this is more likely to be due to an extension of the paired region in the long arm than to increased torsion, specially in species with restricted pairing. *F. acmopetala*, for example, has a higher frequency of bivalents with two *LA* chiasmata in bivalents without a *SA* chiasma:

	Frequencies of chiasmata in the long arm		
	0	1	2
No *SA*-chiasma	0	70·6	29·4
SA-chiasma	3·6	81·8	14·6

Since the first proximal chiasma as a rule is very close to the centro-

mere, the second must be distal to it and therefore due to an extension of pairing rather than to an increased torsion.

7. DISCUSSION

(1) *Contact-point and time-limit.* As a rule pairing starts close to the centromere; exceptions without centric pairing are striking but very rare. From this primary contact-point pairing may extend into the median region. The extent and frequency of this process is determined by the time-limit and is characteristic of each species. In addition to this primary contact-point there is a secondary contact-point which comes into action later than the primary one; thus the action of the secondary contact-point, i.e. the proportion of bivalents with distal pairing, is also determined by the time-limit. The evidence for the secondary contact-point is threefold: Intermittent pairing $(P + D)$ is far more frequent than complete pairing $(P + M + D)$ in species with an early time-limit; in species with a late time-limit, pairing frequencies in the distal region are higher than those in the median region, although the latter has the advantage of greater length in the system of classification of chiasmata; intermittent pachytene pairing has been seen in many species of *Fritillaria* (Darlington, 1935). The assumption of a secondary contact-point follows from the observed regional distribution and from the assumption of a time limit of pairing: The most consistent explanation of distal exceeding median pairing is that of a difference in the time of initiation of pairing in the two regions. It also follows from Darlington's assumption of a pairing advantage of distal over median parts of long chromosome arms (Darlington, 1936). Distal chiasmata frequently are not terminal; this is in accord with the observed failure of pairing in the extreme distal parts of pachytene chromosomes in *F. imperialis* (Darlington, 1935), although the slight terminalization of distal chiasmata tends to obscure this fact.

Darlington (1936) suggested that the differences in chiasma frequency between M and S bivalents can be explained on the assumption that pairing of the former is delayed in relation to that of the latter. All pairing phases examined in this study show differences which are consistent with this assumption.

The action of the time-limit is then defined as limiting the extension of pairing from the primary, proximal, contact point and determining the frequency of incidence of the secondary, distal, contact-point. Distal pairing is directly correlated with the extension of the time-limit; its frequency, especially in S-bivalents, is a measure of the time-limit.

(2) *Torsion.* It has been shown that species with equal pairing frequencies in the several chromosome regions may have different chiasma frequencies and that these differences largely occur in the centric region, i.e. where pairing starts. The difference in chiasma frequency between *Pa* and *I*, for example, is not only due to different time-limits, but also to different torsion (Table IV). Similarly the wide differences in chiasma frequency between clones of *F. imperialis* (2·6–5·0) are probably due to differential torsion, as suggested by Darlington (1936), in addition to timing differences. Only an estimation of the regional chiasma distribution in these clones could show which of these factors is of greater importance.

(3) *Time-limit and torsion in relation to localization.* The evidence of *SA* pairing in relation to chiasma frequency indicates an inverse relationship between the degree of torsion and the extent of the time-limit. The earlier the interruption of pairing, the greater the torsion in paired regions. There is then a compensation for the loss of pairing time in the increase of torsion. Either combination of pairing elements succeeds in preventing failure of metaphase pairing.

It may be asked whether increased torsion is a natural consequence of a reduced time-limit; whether it is to some extent inherent in it; or whether it is entirely and separately determined. The evidence of interspecific comparison favours the second assumption. *F. pontica* has a time-limit which is later, but a torsion which is lower than that of *F. latifolia*, *F. meleagris* and *F. meleagroides*. On the other hand *F. imperialis* has a higher torsion, though its time-limit is similar to that of *F. pallidiflora*. On the present assumptions, an artificial interruption of pairing on the lines of the work of Barber (1940) on *Uvularia* should present a critical test. If torsion is independent of the time-limit, interruption of pairing in such species as *F. pallidiflora* should not increase the frequency of pairing in the short arm.

An artificial interruption of pairing should provide tests for other assumptions advanced in this paper. It should be able to reduce species with a high degree of pairing to the level of procentrically localized species, with the characteristic chiasma distribution of the latter. If suitably graduated it should reduce distal and median pairing before affecting proximal pairing. It should make it possible accurately to determine the time relationship between pairing in all regions.

(4) *A measure of localization.* A measure of localization must express the chiasma frequency of the region in which chiasmata are concentrated, in proportion to the total chiasma frequency of the particular

class of bivalents. The "localization coefficient" is then the proportion of chiasmata formed in any region where chiasmata are prevailingly formed. There may be a proximal localization coefficient, as in *Fritillaria* or in *Mecostethus*, or a terminal localization coefficient, as in *Chrysochraon*. In organisms with a secondary contact-point it may be useful for classification and comparison to add the proportion of pairing in that region. In the *S* bivalents of the species with localized chiasma formation dealt with in this paper, these data are as follows:

Species	Proximal localization coefficient	Distal pairing coefficient
1. *F. acmopetala*	1·00	0
2. *F. latifolia*	0·94	0·01
3. *F. meleagroides*	0·93	0·02
4. *F. meleagris*	0·91	0·02
5. *F. pontica*	0·88	0·05
6. *F. hispanica*	0·70	0·09

8. Summary

1. This paper is a statistical study of the regional distribution of the chiasmata in eight species of *Fritillaria* with varying degrees of localization and chiasma frequency.

2. Pairing starts almost invariably in the centric region and thence may proceed into the median region. In addition there may be a secondary contact-point in the distal region which acts later than the primary, centric, contact-point. Complete pairing as a rule results from the coincidence of proximal (centric and median) and distal pairing.

3. The frequency of the incidence of the secondary contact-point and the extent of pairing proceeding from either or both contact-points are determined by the time-limit of pairing. The shortest time-limit restricts pairing entirely to the centric region. With a gradual extension of the time-limit the proportion of median and distal pairing increases. The amount of distal pairing is a measure of the time-limit.

4. The proportions of centrically localized and of distally paired bivalents are characteristic of each species. They may be used as a measure of "localization" (localization coefficient) and of "free pairing" (distal pairing coefficient).

5. *M* and *S* bivalents show characteristic differences in respect of all elements of the pairing process. These can be consistently explained on the basis of delayed pairing of *M* bivalents which are the longer.

6. Some species with similar pairing frequencies, therefore similar time-limits, have different chiasma frequencies in the centric region. This is explained on the basis of different torsion in that region.

7. There is a consistent relationship between the proportion of short arm pairing to total chiasma frequency in *S* bivalents on one side and the chiasma frequency of *M* bivalents on the other. Excepting the species with the lowest and the highest chiasma frequencies, this relationship is inverse and almost linear.

8. Bivalents with pairing in the short arm have a higher overall chiasma frequency than those with unpaired short arms.

9. The long arms of bivalents without short-arm-pairing have a higher chiasma frequency than that of bivalents with short-arm-pairing. The distribution of chiasmata shows that this is due to a distal shift of the pairing region in the bivalents without short-arm-pairing.

10. It is suggested that time-limit and torsion are complementary variants in the system of pairing in *Fritillaria*: an early time-limit is compensated by increased torsion, and vice versa. By artificial interruption of pairing it may be possible to test this and other assumptions regarding the action of the prime variables of meiosis.

11. Inversion pairing has been seen in two additional species, *F. latifolia* and *F. imperialis*. The number of species with localized pairing so far recorded as having inversions is three, that of species with non-localized pairing is five.

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THE CAUSAL SEQUENCE OF MEIOSIS

II. CONTACT POINTS AND CROSSING-OVER POTENTIAL IN A TRIPLOID *FRITILLARIA*

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(With Four Text-figures)

1. INTRODUCTION

FRITILLARIA LATIFOLIA has a giant form, *major*, which was collected by Elwes in the Caucasus. The species is diploid and its variant triploid. The importance of this relationship lies in the special properties of chromosome pairing and crossing-over shown by the diploid in common with most of its nearest relatives. Pairing is interrupted and crossing-over is in consequence confined to the regions near the centromere. If the diploid is so restricted, how will the triploid behave?

In triploids there is the same two-by-two pairing at pachytene as in diploids, so that there is the same limit to the length of chromosome that can be paired. Nevertheless, the total amount of crossing-over per nucleus or per configuration is increased. At least so we find in *Tulipa*, *Hyacinthus*, *Fritillaria lanceolata* and *Drosophila*. It so happens that the amount of crossing-over per chromosome remains about the same. And this might suggest that the chromosome itself is the unit of *crossing-over potential*. Such a simple formula however hides the problem rather than explains it. The different configuration at pachytene and, in *Drosophila*, the different distribution of crossing-over warn us that the similarity of total frequency must be due to the compensating effects of several different intermediate processes.

An obvious difference in these intermediate processes is that pairing in the triploid takes place in a larger nucleus with more chromosomes to sort themselves out. We already know that this leads in tetraploids to a reduced chiasma-frequency as compared with diploids, a change which is measured by Upcott's reduction factor (1939 *b*). Each of the three chromosomes of the triploid, on the other hand, has a choice of two partners, which should make the finding of one of them easier. The effect of triploidy on crossing-over should therefore be a balanced one and, from the numerical conditions alone, we could not well predict either an increase or a decrease.

There is yet another intermediate process, however, which might have a positive and predominant effect on the triploid, namely change of

partner. Upcott (1939*a*) has shown that, in *Tulipa* at least, changes of partner are more frequent in triploids than in tetraploids. The odd chromosomes being insaturable, as we may say, seem to provide the most variable sequence of zygotene conditions.

Is the increase in crossing-over of triploids per unit of length paired in fact due to the chromosomes often beginning to pair sooner at a second point? Contact at several points would anchor the chromosome; it would prevent the intercalary parts uncoiling before they have paired and thus releasing the internal torsion, which I assume to be necessary for crossing-over. Two point pairing would reserve the intercalary crossing-over potential; it would increase the frequency of crossing-over and change its distribution.[1]

To test this view a plant with localized chiasmata should be of critical value for distribution as well as frequency can then be compared. Haga (1937*a*, *b*) has described a triploid form of *Paris hexaphylla*, a species with extreme localization of chiasmata near the centromeres. He fails however to take advantage of his material. "The mode of pairing" he concludes "is the same as in diploids, except the number of homologues". His photograph indeed shows that the proximal localization of the diploid is maintained in the triploid. The pairing region evidently remains so short that there are few changes of partner at pachytene, and all the extra chromosomes can appear as univalents at first metaphase, a condition unknown in other autotriploids. Trivalents fail most frequently in the shortest chromosomes.

We have another opportunity in *Fritillaria latifolia*. Frankel (1940) has shown, by classification of the chiasma structure of the diploid and its comparison with other species, that there is usually a single point of contact between the pairing chromosomes, that this point is near the centromere, that pairing is interrupted before it is complete and that pairing itself is slower in the two long *M* chromosomes with median centromeres than in the ten shorter *S*'s with subterminal centromeres. All these are properties characteristic of procentric localization and I shall consequently make use of the conclusions I have reached elsewhere in examining this process in terms of the prime variables of meiosis (1940*b*).

2. GENERAL CHARACTER OF THE TRIPLOID

A first glance at the pollen mother cells of the triploid seems to show little evidence of the procentric localization of the diploid species. This is

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[1] I am fortified in this conclusion by learning that Prof. J. B. S. Haldane has arrived at it independently.

especially true if we take cells with the highest number of chiasmata and the fewest univalents (Fig. 1). The trivalents indeed resemble those of the unlocalized *F. lanceolata* both in the distribution of their chiasmata and in the types of their co-orientation (Darlington, 1936). Cells with fewer chiasmata and more univalents begin to show evidences of incomplete, proximally localized, pairing. Comparison of the cells or configurations with different numbers of chiasmata may thus be taken,
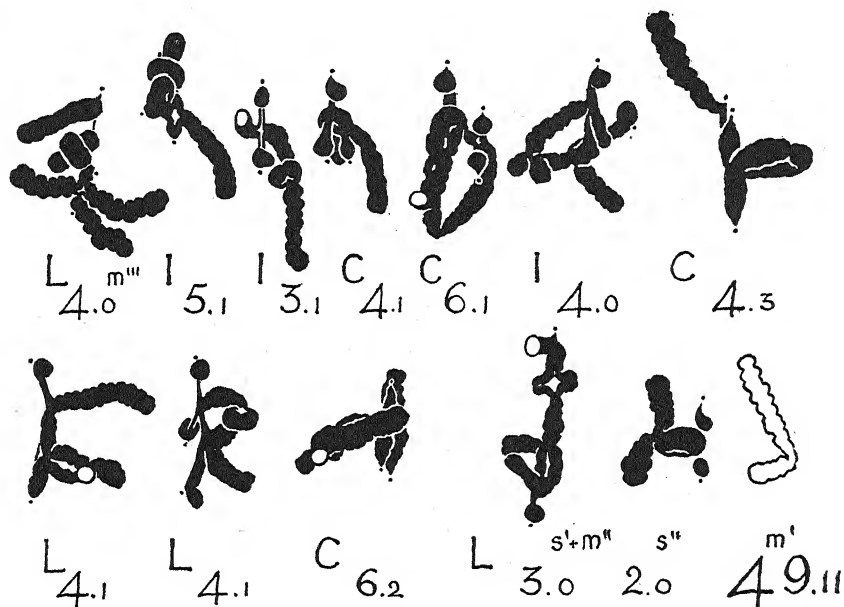

Fig. 1. Complete nucleus of pollen mother-cell of *Fritillaria latifolia major* at first metaphase, with forty-nine chiasmata, eleven terminal (no. 12 in Table II). Numbers of chiasmata under each configuration, co-orientations linear, convergent or indifferent (*L*, *C* or *I*). Eleven trivalents, one being due to illegitimate crossing-over between *M* and *S* chromosomes, not found elsewhere; one *S* bivalent and one *M* univalent. Smear preparation by La Cour, fixed in medium Flemming, stained in gentian violet. × 2000.

as in the diploid, to show stages in the process of pairing, stages at which it can be interrupted. It is on the average interrupted at a stage when far more chiasmata can be formed than in the diploid, yet still not so many as in the triploid of the unlocalized, unrestricted *F. lanceolata*. Hence instead of 0–2 univalents we find 0–8 (Table I).

A record of twelve complete cells, an apparently random sample, gives more precise information. In the first place the chiasma frequency is, as in other triploids, about 50 % greater than that of the corresponding diploid, but retains an equal frequency for *S* and *M* chromosomes

(Table II). Further the proportion of trivalents is slightly higher for the *S* chromosomes (74 %) than for the *M* chromosomes (63 %). The trivalents of both types together have half as many chiasmata again as the bivalents just as in *Tulipa* (Table II). Once more we have an
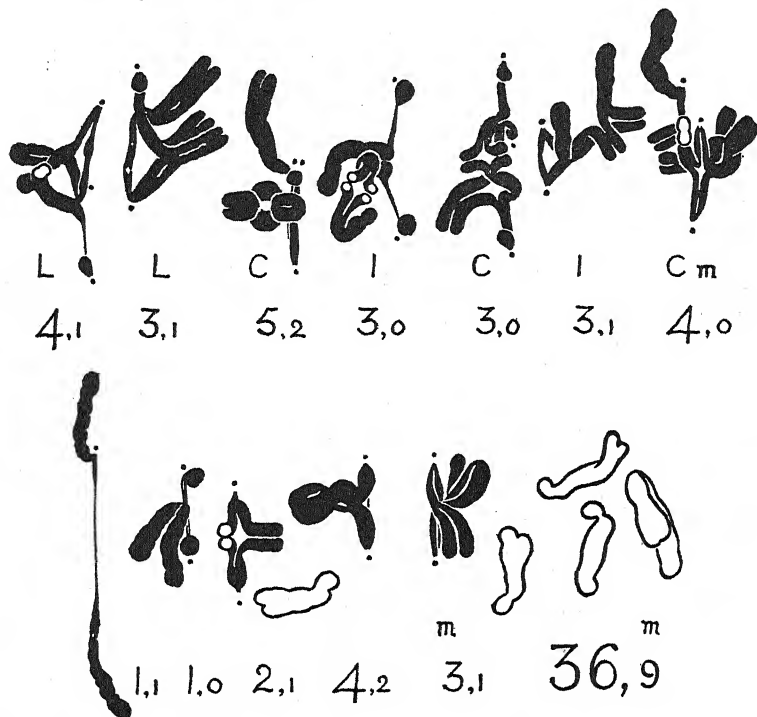
Fig. 2. Nucleus with five bivalents and five univalents (lower row). The chromatid attraction is beginning to lapse at the end of metaphase, but not simultaneously in all parts of the chromosomes (no. 5 in Table II). × 2000.

TABLE I

Frequency of trivalents in thirty-three pollen mother cell nuclei

Trivalents	4	5	6	7	8	9	10	11	12
Nuclei	1	—	2	4	·4	7	6	8	1

Mean per nucleus, 9·1.

indication that the extra chromosome is increasing the crossing-over potential in mere proportion. This can be tested by carrying the analysis a step further.

3. CHANGE OF PARTNER

The configurations can be classified not merely into bivalents as against trivalents but more exactly into configurations derived from pachytene arrangements with 0, 1, 2, 3, or 4 changes of partner. Of

course, these are minimum changes of partner, that is, those still indicated by associations which have led to the formation of chiasmata. Those with no change of partner produce bivalents and univalents, the rest different kinds of trivalents (Fig. 3).

We then find that the M chromosomes never have more than two changes of partner. Their average number is 0·75 as against 1·1 for the S chromosomes (Tables III and IV). We can represent this as a difference between 1·75 and 2·1 effective pairing blocks. It agrees in kind with the difference between M and S types of chromosomes (labelled L and M respectively) in *Hyacinthus* (Stone & Mather, 1932). Here the pairing

TABLE II

First analysis of chiasmata in twelve nuclei of F. latifolia major

No.	Total chiasmata	No. III $(S+M)$	Chiasmata	
			III	II
1	33	7+1=8	29	4
2	34	6+2=8	25	9
3	35	4+0=4	14	20
4	36	8+1=9	28	8
5 D	36	6+1=7	25	11
6	37	9+1=10	31	8
7	37	8+2=10	33	4
8	38	6+1=7	25	13
9	40	8+1=9	32	8
10	42	9+1=10	37	5
11	43	9+2=11	41	2
12 D	49	9+2=11	47	2
Total	461	89+15=104	367	94
Mean	38·2	7·4+1·3=8·7	3·5	2·4

blocks were calculated, not directly from the structure of whole configurations, but indirectly from the curve of variation in the numbers of chiasmata in individual chromosomes. Both results mean that the longer M chromosomes are slower in movement than the shorter S's, a conclusion we reach equally in *Fritillaria* from the equality of their chiasma frequency.

We can next take the numbers of changes of partner in relation to the chiasma frequencies (Table III). Since $n+1$ chiasmata are necessary to reveal n changes of partner, an absence of causal relationship between change of partner and chiasmata would be shown if the lower chiasma classes were merely cut away in the higher change-of-partner classes. We find this true of the M chromosomes. The mean chiasma frequency of M's is almost the same in all change-of-partner classes. The slight increase in the higher classes agrees with our first simple assumption that lower chiasma classes are cut away in these classes. Change of

partner, in so far as it is still demonstrable at metaphase, has no effect on chiasma frequency in the M's. At the same time the fact that chiasma frequency is 50 % higher than that of diploid M's equally in bivalents and trivalents warns us that conditions are different in the triploid.
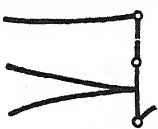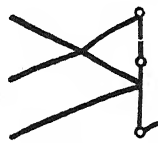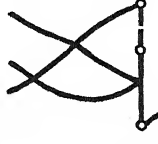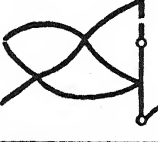
Fig. 3.

Perhaps with a choice of partner in the triploid all pairing is quicker and therefore more effective in storing torsion for crossing over.

The S chromosomes tell a different story. The chiasma frequency increases regularly with the number of changes of partner. But this increase is only in part due to lower chiasma classes being cut off in the higher change-of-partner classes, for the mode shifts with the mean. The

apparent effect of change of partner in increasing crossing-over in the S trivalents therefore seems to be a true and inherent one. Nor are particular instances to be disregarded where they show the limits of variation. One S trivalent with three changes of partner has nine chiasmata, more than twice as many as have been seen in any bivalent of the diploid.

Two conclusions to be drawn from this comparison are of immediate value. First, the most important effect of change of partner on crossing-over occurs with a single change. A second change has less effect. This is easy enough to understand. Anchorage at the two ends has an effect which is not greatly increased by anchorage at a third point.

TABLE III

Relation of chiasma frequency to numbers of changes of partner in S and M configurations (same twelve nuclei)

	Xta	1	2	3	4	5	6	9	Total	Xta	Mean
Change of partner	0	**11**	8	8	4	—	—	—	31	67	*2·16*
	1	×	9	**25**	11	5	1	—	51	168	*3·29*
S	2	×	×	15	**16**	1	1	—	33	120	*3·70*
	3	×	×	×	**2**	1	—	1	4	22	*5·50*
	4	×	×	×	×	1	—	—	1	5	*5·00*
	0	—	3	3	2	1	—	—	9	28	*3·11*
M	1	×	4	3	4	1	—	—	12	38	*3·17*
	2	×	×	2	1	—	—	—	3	10	*3·33*
S, total	—	*11*	*17*	*48*	*33*	*8*	*2*	*1*	*120*	*382*	*3·18*
M, total	—	—	*7*	*8*	*7*	*2*	—	—	*24*	*76*	*3·17*

Secondly the effect of change of partner is different in M and S chromosomes. In fact the absence of effect in M chromosomes verifies its significance in S chromosomes. They act as mutual controls.

For this contrast one or more of the differences between conditions of pairing in M and S chromosomes might be responsible. Of these we already know that pairing is less complete and chiasmata farther apart in the M chromosomes. A second important difference consists in the place where chromosomes change partner. This we must now consider.

4. ORDER OF PAIRING

Changes of partner and chiasmata may be classified by position as well as frequency. They may occur in any of the arbitrary regions into which Frankel and I have divided the chromosomes (Fig. 4). Median and distal changes of partner of course are confined to trivalents with more numerous chiasmata, for pairing which produces few chiasmata is confined as in the diploid to the proximal regions where it starts.

We then find that of the thirty-one S bivalents none have median or distal (M or D) chiasmata only, and relatively few have these in addition to short-arm and proximal (S and P) chiasmata. But amongst

forty-eight trivalents with one change of partner one-fifth of the individual chromosomes have median or distal chiasmata only (Table IVA). The distal associations arise independently only when proximal associations are there already. As in the diploid, proximal contact comes first.

FORMS OF *S* TRIVALENTS WITH SINGLE CHANGES OF PARTNER

C.o.P.	Pachytene inferred	Metaphase observed
S		4 Xta
C		" "
P		" "
M		" "
D		5 Xta

Fig. 4.

In configurations with two changes of partner, individual chromosomes can no longer be identified and we have to consider changes of partner in whole configurations. These show a striking difference, not essentially between configurations with different numbers of changes of

partner, but between those with different numbers of chiasmata. The low chiasma configurations have their changes of partner concentrated in the centric and median regions. The high chiasma configurations have the distribution evened out. This is because the pachytene pairing itself has spread in the high chiasma configurations to the whole length of the chromosomes from the first and second points of contact (Table IV B).

TABLE IV

Segmental distribution of chiasmata (Xta) and changes of partner (C.o.P.) in F. latifolia 3x *(twelve nuclei)*

A. Positions of Xta (individual chromosomes)

C.o.P.		No.	(S) P only	(S) P and MD	MD only	Total
S	0	31^{II}	48	14	—	62
	1	48^{III}	64	52	28	144
M	0	9^{II}	10	8	—	18
	1	12^{III}	16	17	3	36

B. Positions of C.o.P. (whole trivalents)

C.o.P.		Xta	S	C	P	M	D	Total
S	1	2–3	1	13	2	15	2	33
		4–6	2	4	4	3	1	14
		Total	3	17	6	18	3	47
	2	3–6	1	14	16	11	12	54
M	1	2–5	—	9	2	1	—	12

When we look at the positions of change of partner in the two types of chromosome we see that those in the M chromosomes are much more largely in the centric region. The two points of contact have then been on either side of the centromere. They are close together, so close in fact that their effect in anchoring the intercalary torsion is negligible. This, then, is why change of partner has no effect in increasing the chiasma frequency of the M chromosomes.
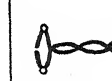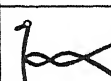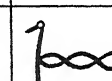
5. BIVALENTS IN THE TRIPLOID

It is not without interest to compare the types of bivalent in the triploid with those found by Frankel in the diploid. Dividing the S bivalents into eight classes, four with and four without short arm chiasmata, we see that the distribution of types is in fact different in two ways.

First, the variance in chiasma-frequency is higher in the triploid bivalents. The M bivalents in the triploid show increase of variance over those of the diploid and at the same time an increase of mean frequency. There can be no doubt that the triploid bivalents retain the essential properties of procentric localization. But they are a selected sample

(29 % of the whole). The process of selection favours some that have been extremely ill-favoured and others that have been extremely well-favoured for pairing. This is not surprising if we assume that distance apart at leptotene determines the earliness and amount of pairing, since, when all three homologues are very far apart, and when two are very close

TABLE V

F. LATIFOLIA : S BIVALENTS IN 2x & 3x					
Xta.	1	2	3	4	T
x A					
mean B					
2x A	2	40	28^t	7	77
2.33 Xta. B	9	12	2^t	–	23
3x A	2	11	11^t	6	30
2.24 Xta. B	15^m	9^{mm}	5^t	2	31
2x T	11	52	30	7	100
3x T	17	20	16	8	61

[t] One bivalent has its distal chiasma nearly terminal.
[m] One bivalent has its proximal chiasma median.
Note. The triploid bivalents in this table and the next include an extra sample beyond those of the twelve cells used in the general analysis. Some discrimination no doubt occurred in selecting these samples but it seems to have reduced the difference between the diploid and the triploid rather than otherwise.

together while the third is far apart, trivalent formation should be equally unlikely. Hence the increase in variance.

Secondly the frequency of chiasmata in the short arm is lower in the triploid bivalents. A chromosome without a chiasma in the short arm has lost the chance of a change of partner in the centric regions; it has lost 34 % of its chance of forming a trivalent.

6: CONTACT-POINTS AND EFFECTIVE TORSION

There remains yet another way of comparing the properties of diploid and triploid. Variance in chiasma frequency per configuration may be taken as evidence of crossing-over mechanism and has been used in this way by Haldane (1931). The variance is low in diploid species of *Fritillaria* with localized chiasmata, with, that is, a single contact point

TABLE VI

F. LATIFOLIA: M BIVALENTS IN 2x & 3x						
Xta	1	2	3	4	5	T
x A			–	–	–	
No. B	–				–	
mean C	–	–				
2x A	2	2	–	–	–	4
20^II B	–	11	4	–	–	15
2.20 Xta C	–	–	–	1	–	1
3x A	1	–	–	–	–	1
13^II B	–	5	2	3	–	10
2.84 Xta C	–	–	1	–	1	2
2x T	2	13	4	1	–	20
3x T	1	5	3	3	1	13

in pairing. In unlocalized species, where we have reason to believe there is often a second distal point of contact, this variance is increased out of proportion to the mean. In the triploid it is again greatly increased. And although the *S* chromosomes have the same chiasma-frequency as the *M*, their variance is differentially affected. It is much higher. Again it is in these chromosomes that the average number of changes of partner is so high. And the number of changes of partner is a minimum index of the number of contact points in pairing.

There is thus a *prima facie* case for regarding the establishment of a second contact point in pairing as a means of increasing the frequency of crossing-over disproportionately to the increase in length of chromosome paired.

This principle if true must lie at the root of the mechanics of crossing-over in diploids as well as polyploids. It must also have immediate application to the understanding of various kinds of "asynapsis". But

TABLE VII

Relation of the number of effective pairing blocks to the variance in chiasma frequency per configuration (derived from Tables III, V and VI)

F. latifolia		Chiasma frequency			Mean effective pairing blocks (= changes of partner + 1)
		Mean	Variance	V/M	
2x	M	2·20	0·48	**0·22**	*ca.* 1·1
	S	2·33	0·59	**0·25**	*ca.* 1·1
3x	M	3·17	0·93	**0·30**	1·75
	S	3·18	1·81	**0·57**	2·10

I would not suggest that its proof will be easy or its operation unconditional. If torsion is retained by intermittent contact, the effect of its retention will be greatly affected by the distance of the two contact points apart and by the extent to which the intercalary regions eventually pair. We already see that changes of partner in the *M*'s of our triploid are too close together to have any effect, and we know that a large amount of stored torsion may be wasted in parts of chromosomes which never pair in the diploid *F. Elwesii*.

In order to test this principle we shall therefore need to carry out a similar analysis of other triploids and tetraploids with different types of chromosomes and different conditions of pairing.

SUMMARY

1. *Fritillaria latifolia major* is a triploid form of a diploid species with chiasmata localized near the centromere.

2. The triploid has 3·2 chiasmata per configuration as against 2·3 in the diploid.

3. It has an average of 9·1 trivalents out of 12 possible per cell. The trivalents have an average of 3·5 as against the 2·4 chiasmata of bivalents.

4. *M* chromosomes have the same chiasma frequency as *S* (3·17 against 3·18), whereas in the diploid they have a slightly lower frequency (2·20 as against 2·33).

5. Bivalents in the diploid never have more than four chiasmata and these are always concentrated near the centromere. Configurations in the triploid have as many as nine chiasmata and those with more than four chiasmata show no localization. Those with fewer chiasmata, and especially bivalents, show proximal localization.

6. M chromosomes have an average of 0·75 changes of partner shown by chiasma relationships, S an average of 1·10, i.e. 1·75 and 2·10 effective pairing blocks. Thus M's move less freely than S's at zygotene and have fewer trivalents at metaphase (63 % against 74 %).

7. The chiasma frequency of S chromosomes is higher in configurations with more changes of partner, that is, with more contact points at zygotene (Table III). Its coefficient of variation changes in the same way (Table VII).

8. In M chromosomes the second contact point has no effect on the frequency of crossing-over, which is uniformly higher than in the diploid, probably because pairing is made easier by the choice of partner.

9. The difference in effect of change of partner in M and S is to be correlated with the positions of the points of contact, which are close together in M, farther apart in S. The effect of two contacts thus depends on the length of segment between them.

10. This effect of two points of contact on the intercalary segment can consist only in their preventing its uncoiling. Such anchorage would preserve the crossing-over potential. The number of contact points is higher in triploids than in diploids, and this difference will therefore account for the increase and change of distribution found in their crossing-over.

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APPENDIX

The chromosomes of the triploid *Tulipa praecox* described by Upcott are similar in shape to the *S* chromosomes of *Fritillaria.* It therefore seems worth while to compare the behaviour of the two in regard to the relationship of changes of partner and chiasma frequency. *T. praecox* has slightly smaller chromosomes with a slightly lower average chiasma frequency (2·90 against 3·18) but about the same average frequency of change of partner (1·15 against 1·10).

Table VIII shows the position in *Tulipa praecox.* The effect of change of partner is similar. The increase of chiasma frequency between the no-change and one-change classes is 0·84 against 1·13 in *Fritillaria.* The tulip is closer to the limits of its crossing-over potential in the no-change class and the effect of a single change of partner is less pronounced. The effect of a second change is negligible. The increase seems to be no more than the inherent selection of the sample requires. Probably beyond a certain frequency per unit of length changes of partner will militate against completeness of pairing at pachytene. That frequency will be lower in *Tulipa* than in *Fritillaria.*

TABLE VIII

Chiasma frequencies of configurations with different numbers of changes of partner in twenty cells of Tulipa praecox *(3x = 36). Seven configurations include six chromosomes owing to interchange and have four to six changes of partner (after Upcott, 1939 a)*

Xta	0	1	2	3	4	5	6	7	8	9	T	M
0	—	8	**21**	8	—	—	—	—	—	—	37	*2·00*
1	×	×	40	**70**	19	—	—	—	—	—	129	*2·84*
2	×	×	×	**40**	14	1	—	—	—	—	55	*3·30*
3	×	×	×	×	**5**	—	—	—	—	—	5	*4·00*
4	×	×	×	×	×	—	1	1	1	—	3	*7·00*
5	×	×	×	×	×	×	—	—	1	1	2	*8·50*
6	×	×	×	×	×	×	×	—	—	2	2	*9·00*
T	—	8	61	118	38	1	1	1	2	3	240	*2·90*

THE CAUSAL SEQUENCE OF MEIOSIS

III. THE EFFECT OF HYBRIDITY ON MALE AND FEMALE CELLS IN *LILIUM*

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(With Eighteen Text-figures)

CONTENTS

1. INTRODUCTION

MEIOSIS in female cells, eggs or embryo-sac mother cells, inevitably differs from that in male cells on account of the difference in living space available for the chromosomes. Comparison, however, has been neglected in the past. The technical difficulties of studying meiosis in the solitary female cells have usually been a sufficient deterrent. Embryo-sac divisions now present several problems that call for solution. Zygotene pairing, crossing-over, co-orientation and the abnormal consequences of hybridity all demand comparison with the conditions in male cells, comparison at once of their mechanical conditions and their genetical consequences.

We have therefore examined meiosis in the embryo-sac mother cells of various species and hybrids, diploid and polyploid, in *Lilium*, *Fritillaria* and *Tulipa*, the group with which we are best acquainted. The results, in pre-anaphase stages, show a closer similarity of behaviour of male and female cells than we had expected. Particularly important seems to be the adjustment in localization of chiasmata to correspond in the two sexes in *Fritillaria*.

Our present object is to compare merely the metaphase configurations in embryo-sac and pollen mother cells of *Lilium testaceum*. This plant is reputed to be a first cross between *L. candidum* and *L. chalcedonicum*. Pollen meiosis has been described by Ribbands (1937). He has shown that this cross, like *L. Marhan*, is hybrid for numerous inversions. These

act on meiosis in two ways. Where they pair and cross-over bridges are formed at anaphase. Where they fail to pair chiasma formation is reduced. Statistical records show a combination of these two effects.

The development of the study however has led us to re-examine the pollen mother cells for special statistical treatment. This treatment is in several respects novel and perhaps requires some apology. The embryo-sac sections are remarkable in that, owing to the spacious arrangement of the chromosomes, every complete nucleus can be, and has been, analysed and recorded—thirty-two metaphases in twenty-four slides. The pollen smears on the other hand are subject to discrimination. A proportion of cells cannot be recorded owing to various conditions—damage, diagonal orientation or complexity of configuration. These conditions may be mixed, hence a discrimination may arise which will lead to an error of sampling. It is worth while comparing the perfect sample of female cells with Ribbands' slightly selected sample of male cells. On the other hand the enormous numbers of male cells available enable us to use discrimination for the special purpose of studying corre-lations. This is necessary where the range of variation of both variables falls in an extremely skew curve. The discrimination we have used con-sisted in taking pollen mother cells more evenly distributed over the range of their total chiasma frequency (and therefore having a lower mean) than a sample of natural distribution would allow. This enables us to make a significant comparison of the properties of the two types of chromosomes, M and S, at the two extremes of their behaviour. In this regard we are indebted to Dr K. Mather, Dr M. M. Richardson and Mr C. R. Ribbands for the use of their unpublished data on this and other species and hybrids.

2. TECHNIQUE

The embryo-sac presents a special problem of fixation on account of the thickness of the ovary wall. The wall itself can be satisfactorily pre-pared by the smear method for the study of mitosis, for which purpose it provides a useful substitute for root-tips. This method is not, however, practicable for the embryo-sacs. They are too few in number and too vulnerable by reason of their size.

A special method of dissection was therefore used. By taking advan-tage of the vascular fibres running lengthwise it is possible to peel rows of ovules off the larger liliaceous ovaries with a sharp scalpel. These ovules remain together until they are embedded. They can then be cut in suitable groups.

Even with this method it is necessary to use a fixative of high penetrance. The following formula, a modification of solution 2BE with increased osmic and acetic acid, gave as fine a fixation as any used with pollen mother cells. We shall refer to it as 2BX:

18·0 c.c. 1 % chromic acid.   9·0 c.c. water.

11·0 c.c. 2 % osmic acid.   0·2 g. potassium bichromate.

10·0 c.c. 5 % acetic acid.   0·01 g. saponine.

The sections need to be cut at 40μ. In doing this two precautions are necessary. First, the slide, smeared with albumen, should be heated before mounting to help the adhesion of the thick ribbon. Secondly, a weak solution of gentian violet, 0·1 % or one-tenth saturated, should be used to allow of easier differentiation.

The pollen mother-cells were fixed in 2BE solution and hydrolysed 8 min. at 60° C. in N hydrochloric acid. They were stained by the Feulgen method.

TABLE I

*Chiasma-frequencies of* Lilium testaceum, ♂ *and* ♀, *and of* L. candidum ♂ (*Mather*)

Chiasmata		0	1	2	3	4	5	6	7	II	Xta	M	V
L. testaceum, ♂	M	15	19	13	14	6	1	—	—	68	116	1·71	1·82
	S	58	85	92	58	34	11	1	1	340	647	1·90	1·92
L. testaceum, ♀	M	6	8	13	11	14	8	3	1	64	188	2·94	3·0
	S	28	49	92	79	62	8	2	—	320	770	2·41	1·7
L. candidum, ♂	M	—	—	—	1	5	4	1	1	12	56	4·67	1·15
	S	—	4	10	28	12	6	—	—	60	186	3·10	1·04

3. EMBRYO-SAC MOTHER CELLS

The arrangement of the bivalents on the first metaphase plate is slower than in the pollen mother-cells, no doubt on account of the cells being larger. We see bivalents lying far off the plate, unco-orientated when their fellows are already finally arranged. Nor is this always due to remoteness of the two centromeres (Figs. 1, 5). Another difference from the male cells is that the spindle is larger. Since the poles are farther apart it is not surprising that the co-orientated centromeres themselves move farther apart before the lapse of chromatid attractions which liberates the chromosomes for their anaphase movement. Hence the extreme tension of the proximal segments (Figs. 3, 4).

A first review of these cells shows two general properties distinguishing them from those of *Lilium* species: the lower mean chiasma frequency and its higher variance (Table I). Some chromosomes fail to
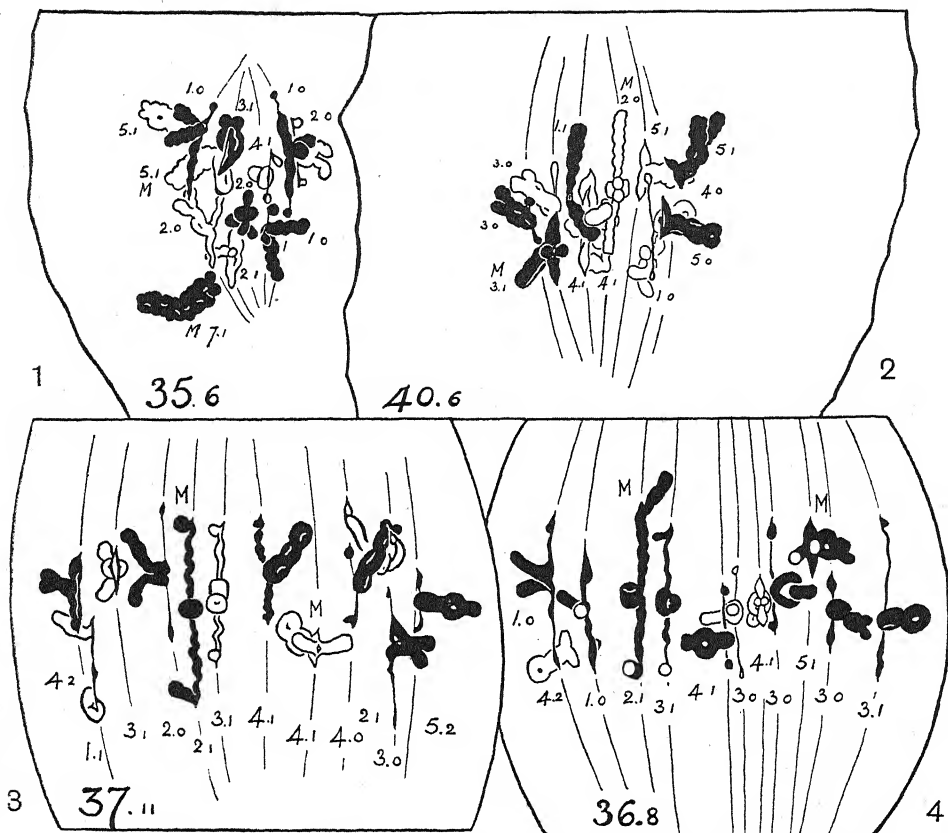
Figs. 1–4. First metaphase with twelve bivalents in the embryo sac of *Lilium testaceum*, giving total and terminal chiasmata under each bivalent and nucleus. *M*, the two bivalents with median centromeres. Figs. 1 and 2 *in situ*; Figs. 3 and 4 spaced. ×1600.
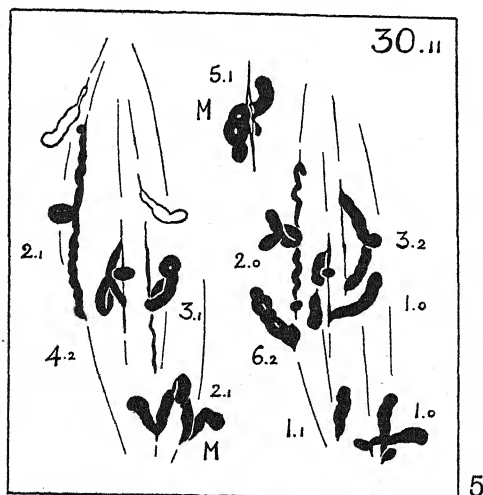
Fig. 5. First metaphase in the embryo sac mother cell, showing eleven bivalents, two of them non-co-orientated. Drawn at three levels separately. ×1600.

form chiasmata and are unpaired at metaphase. These univalents lie distributed at random on either side of the plate. They do not show the position correlation which Ribbands described in the male cells.
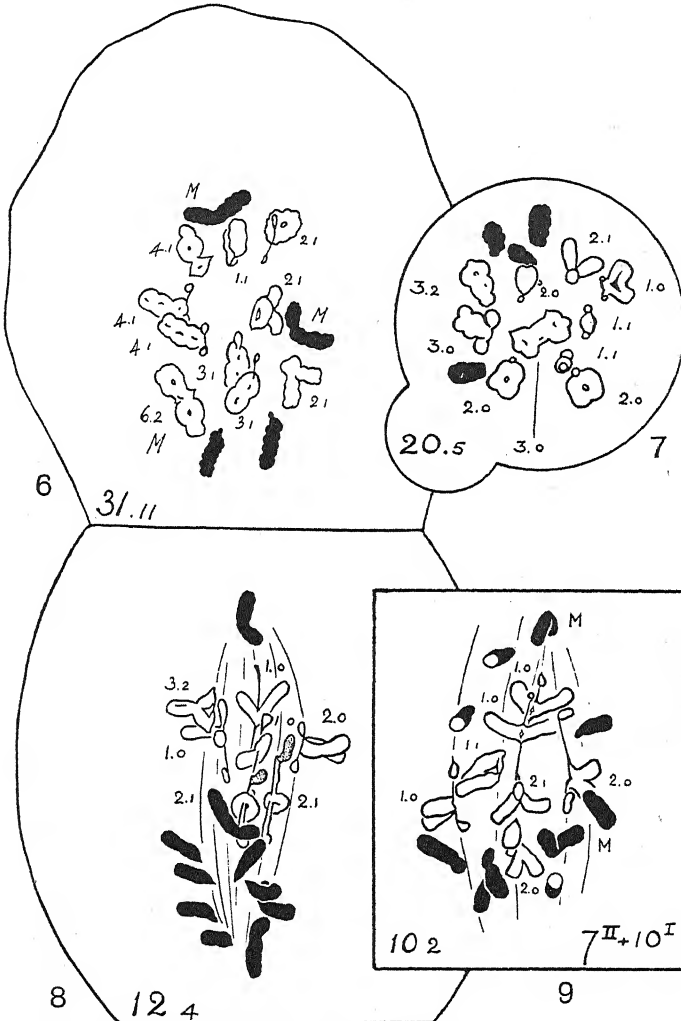
Figs. 6–9. First metaphase in four embryo-sacs with decreasing numbers of chiasmata, increasing proximal localization and frequency of univalents. Fig. 7 shows one inversion chiasma. × 1700.

A further study shows that the reduced numbers of chiasmata are distinctly grouped or localized. The two regions that are favoured are proximal and distal. An even distribution of chiasmata is reached only

when the longer chromosome arms have four or more chiasmata—and
then they are always even. The long arm of the shortest S chromosomes
can attain an even distribution with three chiasmata. This distribution
corresponds with the "complete pairing" condition described by Frankel
in *Fritillaria*.

If we turn to special cells, those with the fewest chiasmata, this
impression is deepened. In the extreme examples illustrated (Fig. 9) we
see in fact that every bivalent has one or two chiasmata close to the
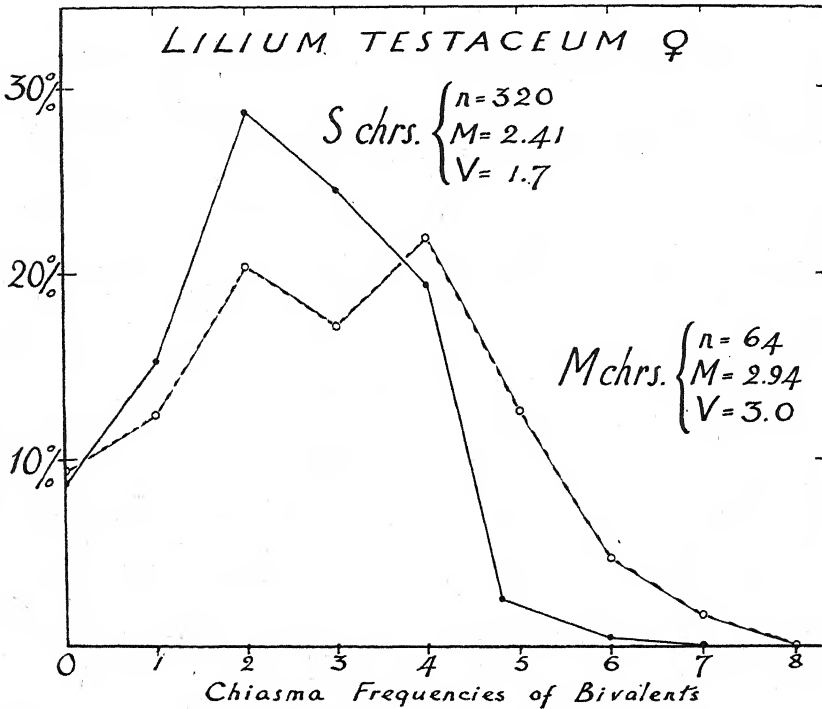
Fig. 10. Chiasma frequencies in the sample of thirty-two embryo-sac mother cells.

centromere. These bivalents might be taken from a typical cell of
Fritillaria Meleagris showing procentric localization.

Following the argument developed elsewhere (Darlington, 1940 *b*) that
different degrees of chiasma frequency correspond to different stages at
which pairing has been interrupted, the conclusion must be reached that
in this plant most of the chromosomes begin to pair near the centromere
while the rest begin to pair near the distal end of the chromosome.

In order to test this view more thoroughly it is necessary to turn to
the pollen mother cells, where we can pick out a larger sample of cells

with the lower chiasma-frequencies which are the more significant for this purpose.

TABLE II

Comparison of pairing and chiasma frequencies in ♀ L. testaceum, *in selected samples and unselected (Ribbands) of ♂ L.* testaceum, *in ♂ L. Marhan (Richardson) and in ♂ L. candidum (Mather)*

Species	Mean no. of Xta per bivalent			Mean no. of univalents per cell			Chance of pairing	
	M	*S*	Total	*M* (2)	*S* (10)	Total	*M*	*S*
L. testaceum, ♂*	1·71	1·90	*1·87*	0·44	1·71	*2·15*	0·78	0·83
L. Marhan	1·88	2·26	*2·20*	0·18	0·52	*0·70*	0·91	0·95
L. testaceum, ♀*	2·94	2·41	*2·50*	0·18	0·88	*1·06*	0·91	0·91
L. testaceum, ♂	—	—	*2·61*	—	—	*0·68*	—	—
L. candidum	4·7	3·1	*3·30*	—	—	*0·00*	1·00	1·00

* Present observations.

4. POLLEN MOTHER CELLS

The kind of sample taken for study can be seen by the comparison of univalent and chiasma frequencies (Table II and Fig. 11). In the natural

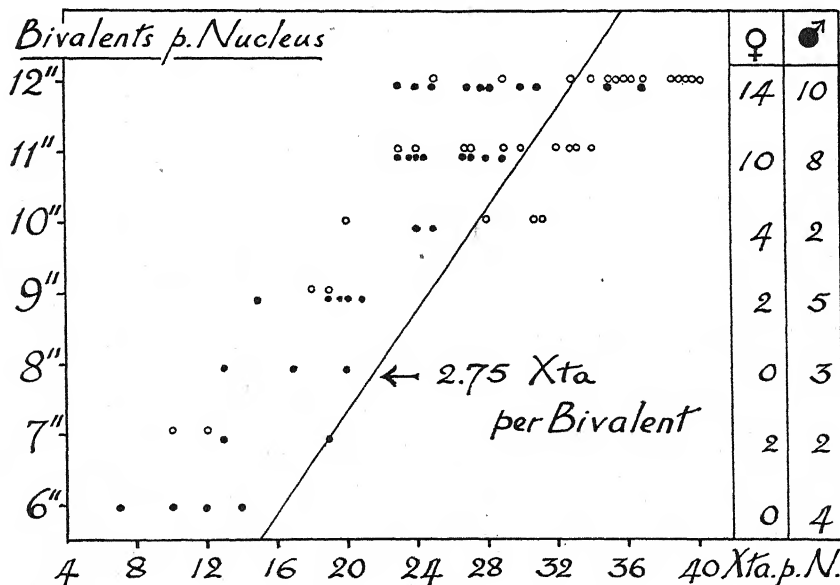Fig. 11. Record of the chiasma frequency of nuclei with different numbers of bivalents in the two samples of *L. testaceum*, showing lower frequency per bivalent in the lower cells.

sample of female meiosis nearly half the cells have twelve bivalents. Further the chiasma frequency of these bivalents is higher than that in

the cells with fewer bivalents. This follows the natural law that univalents, being in origin bivalents-with-no-chiasmata, the frequency of this class is related to the frequency of the classes with one, two or more chiasmata. In the selected male sample the frequency of chiasmata in bivalents remains nearly the same in all classes and we have chosen disproportionately many cells with large numbers of univalents.

In the thirty-four cells selected we see at once the same principle of localization, both proximal and distal, as in the female cells (Fig. 12).
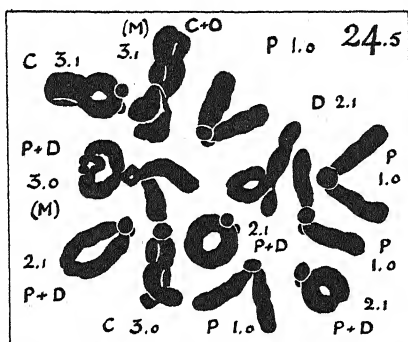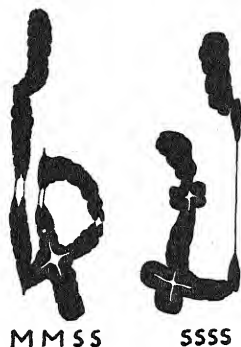
Fig. 12.   Fig. 13.

Fig. 12. Pollen mother cell of *L. testaceum* in polar view, showing frequency and localized distribution of chiasmata; *P*, proximal; *D*, distal; *C*, complete or even. × 1600.

Fig. 13. Two translocation groups, each showing an inversion chiasma giving acentric and dicentric chromatids. Parallel co-orientations. × 1600.

When these cells are arranged in order of diminishing chiasma-frequency we see the effects of this localization increasing in a diagrammatic way (Figs. 14, 15). In the higher cells a few bivalents are found with chiasmata grouped in the middle of an arm (intermediately) but this type disappears in the lowest cells, as does the type with complete pairing, having four to seven chiasmata.

In order to make the most of these observations we need, however arbitrarily, to record the points of pairing and examine their relation to the total chiasma frequency and to the special behaviour of the *M* chromosomes (with median centromeres). We can divide thirty-three of our cells into three equal samples with different numbers of total chiasmata. We then arrive at the classification shown in Figs. 16 and 17.

It now appears that these classes can be represented without exception as corresponding to stages in the completion of pairing. No-pairing passes into one-point pairing—proximal, distal or rarely intermediate—and this in turn passes, perhaps directly, into complete-pairing,

or more probably into two-point pairing which itself can later become complete.

Between the M and S chromosomes two differences appear. In the first place the S series seem to represent later stages than the corresponding M series. It is as though the S chromosomes were quicker in getting to work and in consequence reached completion earlier on the average and consequently at each corresponding stage more frequently. In the second place the S chromosomes show more proximal and less distal pairing than the M chromosomes.

These last observations must be considered together and in relation to the different effects of proximal contact on M and S chromosomes. In *Fritillaria*, where the pairing regularly begins near the centromere, the S chromosomes always have a chiasma frequency equal to, or higher than, that of the longer M's (Frankel, 1940). *L. testaceum* is intermediate in this respect between *Lilium* and *Fritillaria* species (Darlington, 1940a). Apparently so far as the pairing begins at the centromere the M chromosomes suffer from its interruption more than the S's. And for this mechanical reason that the centromere of an M is in the middle of the chromosome and therefore doubly tethered, that of an S is near an end and therefore only singly tethered.

Returning to our *Lilium*, where we see that the point of first contact is optional, we should expect (a) that the M's would suffer less in pairing than those of *Fritillaria*, where proximal contact is obligatory, and (b) that the distal contact should be relatively more important in the M's than in the S's. Both of these expectations are borne out. *Lilium testaceum* is intermediate between the *Lilium* and *Fritillaria* species in the M/S chiasma frequency proportions (as shown by the true female sample), and its M chromosomes have relatively more distal contacts than its S (as shown by all classes of the male sample).

Before considering this any further, let us note that the absolute as well as the relative frequency of distal contacts is less in the S chromosomes than in the M. This may be due in part to some distal contacts having been lost in the two-point and complete classes. But in part it must also be due to a real reduction. Pairing at one end has reduced the chance of pairing at the other. The low frequency of the two-point class in all groups supports this view.

The relative frequencies of chiasmata in M and S chromosomes may be profitably analysed in another way. We can co-ordinate the total M and S chiasma frequencies of separate nuclei in a single graph (Fig. 18). For this purpose the combination of the male and female samples is

Fig. 14.

Fig. 15.

Figs. 14 and 15. Series of complete cells of male *L. testaceum* with decreasing chiasma frequencies, corresponding to Figs. 6–9 for the embryo sacs. These figures show the method of scoring used in Table III and Figs. 16 and 17.
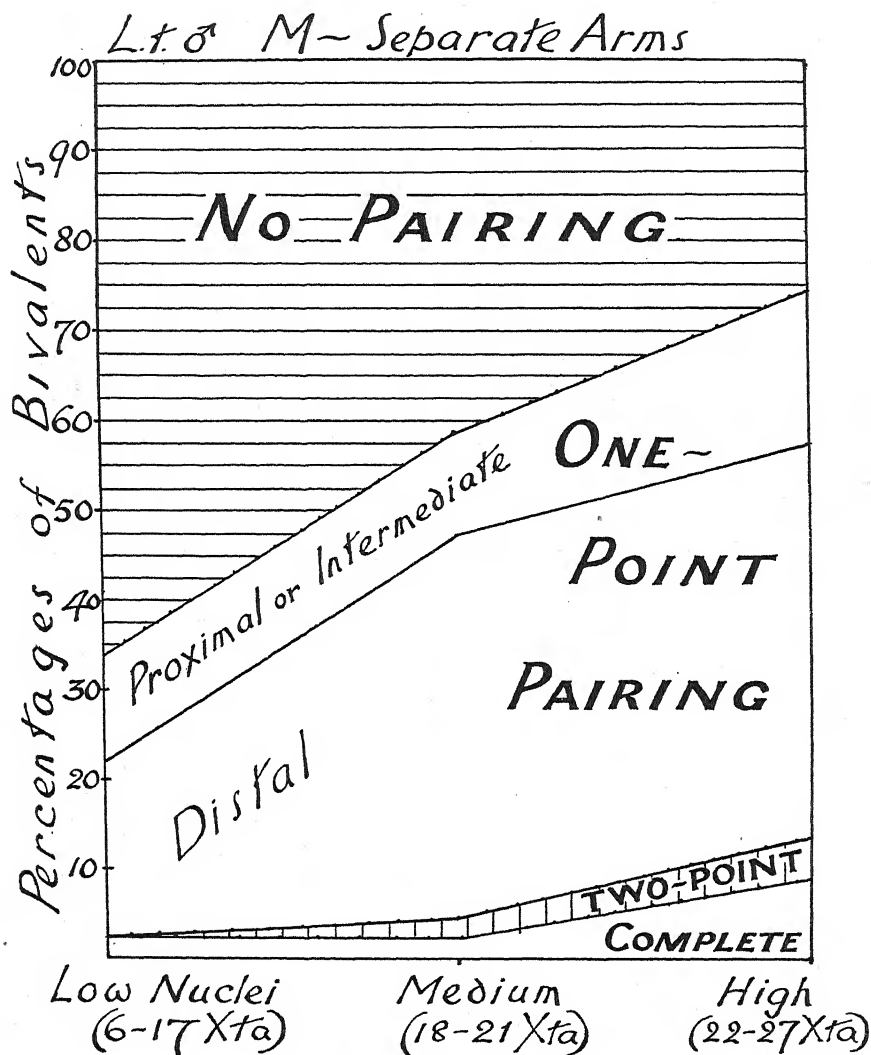
Fig. 16.

useful, for although the male sample was selected it was not selected for differential properties of *M* and *S*. We find in fact that the two samples agree in demonstrating the expected principle, namely that the *M* chiasmata fall away more rapidly than the *S* chiasmata. The regression line of *M* on *S* evidently crosses the direct proportionality line given in the figure at a considerable angle.
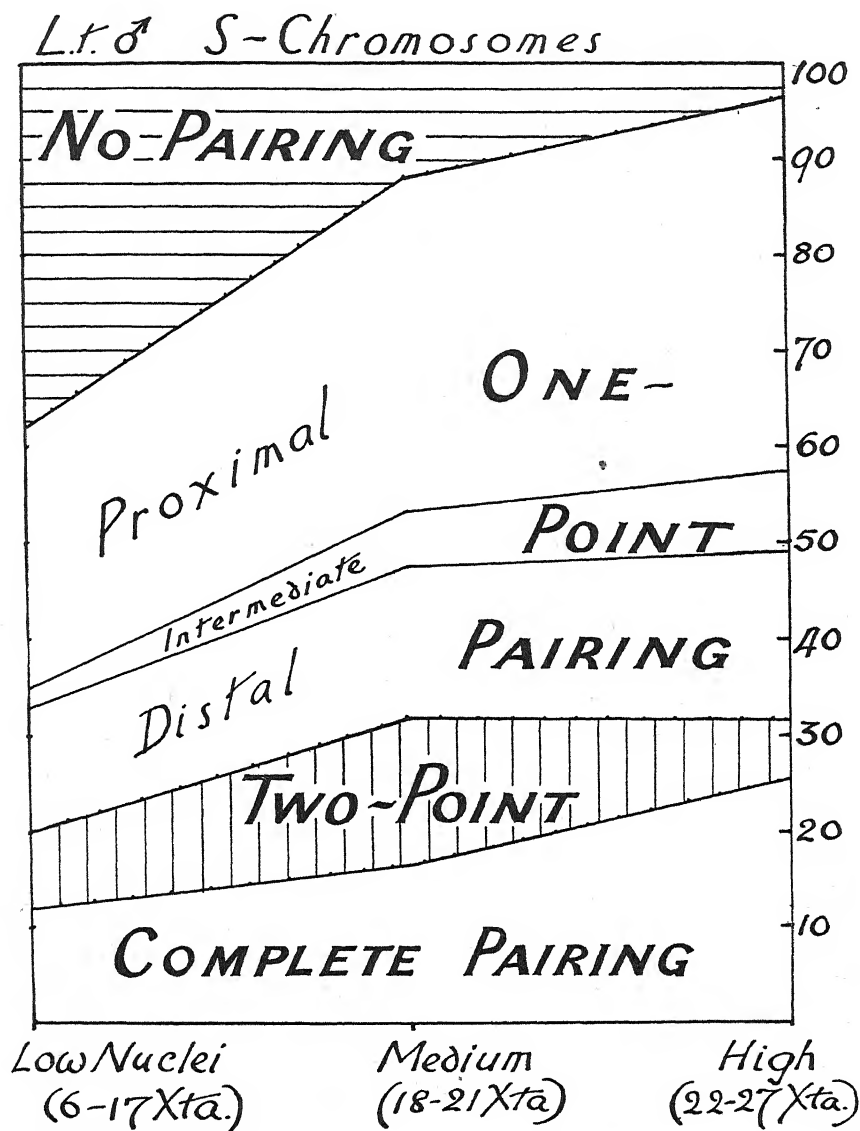
Fig. 17.
Figs. 16 and 17. Graphs representing Table III and showing developmental relationship of
M and *S* chromosomes in low and high chiasma nuclei.

This relationship enables us to understand another of the differences
between the *Lilium* hybrid and its parent species. If we take those cells
of the hybrid which correspond in total chiasma frequency with the

species we find that the M chromosomes have a higher chiasma frequency than the S. The upper end of the hybrid sample corresponds with a

TABLE III

Analysis of pairing points in Lilium testaceum ♂ *bivalents taking low (6–17), medium (18–21) and high (22–27) chiasma nuclei separately (cf. graphs, Figs.* 16, 17)

	Pairing points	None	One P, D or I	Two $P+D$	Three (2 arms)	Complete	Total
S	Low	42	46	9	—	13	110
	Medium	13	62	17	—	18	110
	High	3	73	6	—	28	110
M, one arm	Low	29	14	—	—	1	44
	Medium	17	25	1	—	1	44
	High	11	28	1	—	4	44
M, both arms	Low	9	11	2	—	—	22
	Medium	5	7	9	1	—	22
	High	1	9	10	1	1	22
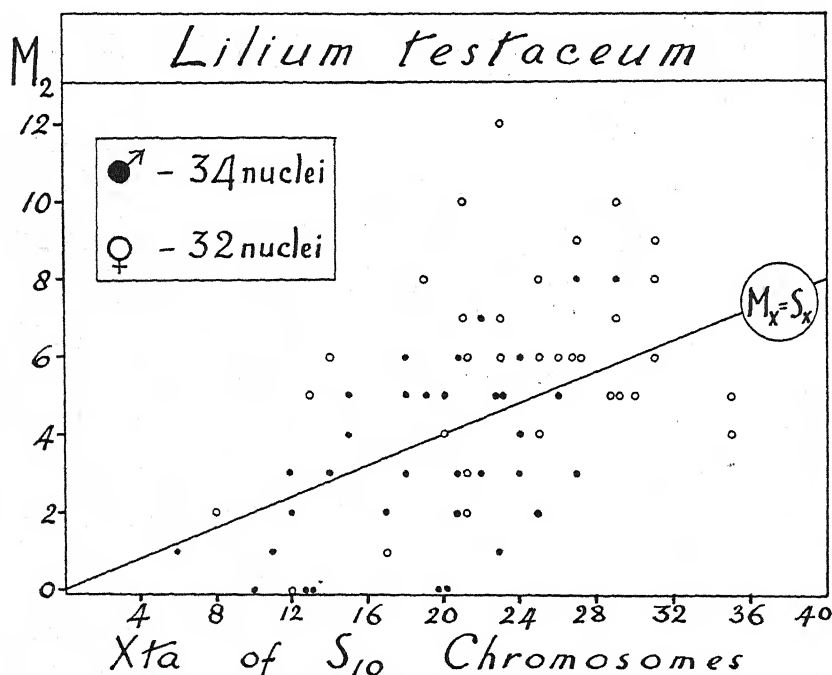
Fig. 18. Chiasma frequencies of M and S chromosomes co-ordinated in the same nuclei of male and female cells, showing indirect variation.

species sample. The lower end is responsible for the divergence between the two at once in the absolute frequency, relative frequency, and spatial

distribution of chiasmata. Expressing these differences in terms of the rate of pairing, we may say that pairing proceeds at a more variable rate in the hybrid and its average rate is lower.

Why should these differences go together? Variations in rate of pairing and chiasma frequency in the species are no doubt due to chance variations in the spacing of the leptotene chromosomes. In the hybrid a second source of variation is added. The homologous chromosomes differ in regard to their genetic structure, chiefly owing to inversions but partly also owing to translocations (Fig. 13; cf. Ribbands, 1937). These dislocations must interrupt the zip-process of pairing and, demanding a new point of contact, they will introduce a new obstacle and a new source of variation to the process of pairing depending on the choice of end at which the first contact is made. The obstacle and the variation are in fact what our statistical analysis and its timing interpretation require.

In *Lilium testaceum*, as in the triploid *Fritillaria*, we can now see the results of applying a more searching statistical analysis to the study of the frequencies of chiasmata. By taking these frequencies not as an undifferentiated mass but separated in different classes of bivalents and different classes of nuclei, we can represent a sample of cells not as a static picture of the results of undefined processes of development but as a dynamic picture which by its own movement defines these processes and reveals causal relationships between them that would evade any more direct mode of attack.

5. CONCLUSION

The first and least disputable conclusion to be drawn from this treatment is that the samples on which our ordinary observations of meiosis depend consist of cells which differ in a series of correlated respects affecting different types of chromosomes differently.

The second conclusion which seems to follow is that these correlations are due to a unitary control in the co-ordination of the chromosomes in each cell; further that the differences in properties between the different types of chromosomes (*M* and *S*) are such as would arise if they had different rates of movements in the cells, while the differences between the different classes of nuclei are such as would arise if different periods were available for movement.

The third conclusion, which follows immediately from the last, is that the co-ordination is a co-ordination of the time during which the pairing of the chromosomes can take place. The pairing is slower or the time shorter in the crosses than in the species.

Our knowledge of the structural differences between the chromosomes that have to pair in *Lilium testaceum* leaves no doubt that their failure is due to the process being retarded by these obstacles. This is probably true of most species crosses, but in those where the pairing is largely suppressed we may well suppose that the time limit has come into play earlier. We have no reason to suppose that the new balance of a species cross will give the same accurate adjustment as allows for the regular meiosis of its parents.

6. SUMMARY

1. The frequency of chiasmata and of univalents is similar in pollen and embryo sac mother cells of *Lilium testaceum*.

2. Cells with fewest chiasmata have those chiasmata most strongly localized either proximally or distally.

3. Hence pairing must have begun either near the centromere or near an end. The contact point is optional.

4. *M* chromosomes come into contact more readily near the ends, *S* chromosomes near the centromere.

5. But *M* chromosomes pair more slowly, so that the frequency of their chiasmata falls away more rapidly in low chiasma cells than that of *S*'s.

6. Samples of cells used for the study of meiosis in species and crosses must therefore be regarded as representing cross-sections of the process of pairing secured by a variable interruption of the process. Statistical treatment can be used to indicate the order of pairing and the means of interruption.

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THE GENETICS OF *VERBENA*

II. CHEMISTRY OF THE FLOWER COLOUR VARIATIONS

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CONTENTS

1. INTRODUCTION

FLOWER colour in the garden *Verbena* hybrids is affected by at least four pairs of mendelian factors and two groups of three multiple allelomorphs (Beale, 1940). In the present paper the chemical nature of the differences between the various colour types will be considered. For general reviews of the genetics and chemistry of flower-colour variation, reference should be made to the papers of Scott-Moncrieff (1936) and Lawrence & Price (1940).

2. METHODS

(i) *Identification of anthocyanins*

The anthocyanins were tested by the qualitative methods of Robinson & Robinson (1931, 1932, 1933). The purple and maroon anthocyanins (delphinidin 3 : 5-dimonoside and delphinidin 3-monoside respectively) have also been crystallized and fully identified by Scott-Moncrieff & Sturgess (1940).

It was impracticable to test fully some hundreds of samples, such as would occur in a large F_2 family. Consequently, when a large number of plants was to be tested, only a few of the (1 % hydrochloric acid) extracts were hydrolysed; the remainder were merely saturated with sodium chloride, amyl alcohol added and the solution shaken. When it was known that only one of the four anthocyanins—delphinidin 3 : 5-dimonoside, delphinidin 3-monoside, pelargonidin 3 : 5-dimonoside and pelargonidin 3-monoside—was likely to be present, the pigment could be readily identified by this method, because monosides are extracted by amyl alcohol while dimonosides are not, and pelargonidin derivatives give a much redder solution than those of delphinidin.

(ii) *Tests for acylation of anthocyanins*

The presence of acylated (complex) anthocyanins was suspected when the distribution number was found to be greater for fresh extracts than for those which had been kept for some weeks.

1 % aqueous hydrochloric acid solutions of *purple* and *scarlet-magenta* flowers, freshly extracted (i.e. less than 24 hr. previously), were saturated with sodium chloride and shaken with amyl alcohol; a considerable proportion of the pigment was seen to be transferred to the alcohol. After 3 weeks the same extracts were tested and the distribution numbers then found to be nil, indicating removal of the acyl groups.

The anthocyanins from *plum* and *plum-purple* flowers differed from all others in that not a trace of pigment was extracted by amyl alcohol, even when fresh extracts, saturated with sodium chloride, were used. Hence the anthocyanins in plum and plum-purple flowers are not acylated.

The anthocyanin in *scarlet* flowers is also probably acylated, since on shaking up the fresh solution with amyl alcohol, the distribution number was found to be higher than is usual for pelargonidin 3-monoside.

Further tests were made on fresh extracts of the *scarlet-magenta* and *purple* pigments. The solutions were heated to boiling-point, excess of 10 % aqueous sodium hydroxide added, and the mixtures boiled for 30 sec., cooled and made just acid. They were then saturated with sodium chloride and shaken with amyl alcohol, when the distribution numbers were found to be zero, indicating that the acyl group or groups had been removed.

(iii) pH *determinations*

For the pH measurements, seventy flowers were taken in each sample, the corolla-tubes removed, the petal-lobes ground up, distilled water

added and the pH of the solution estimated by means of a glass electrode. Three samples of plum and three of scarlet-magenta flowers were tested. The results obtained were consistent to one place of decimals, giving for plum a pH value of 5·8 and for scarlet-magenta 5·4.

(iv) *Tests for copigmentation*

Copigmentation of the anthocyanins was demonstrated by the following method. The samples to be tested, previously shown to contain the same anthocyanin, were extracted with 1 % hydrochloric acid and the solutions diluted to approximately the same strength. The copigmented solutions were bluer, and on heating, this increased blueness disappeared, but reappeared on cooling. The colour of the uncopigmented solutions was unaffected by heating.

The presence of anthoxanthins (which may or may not act as copigments) was demonstrated by extracting the aqueous acid solution with amyl alcohol or ethyl acetate and shaking up the extract with dilute sodium hydroxide solution. Using the copigmented solution, a deep yellow colour was produced in the sodium hydroxide; with the uncopigmented solution, only a very pale yellow was obtained. Hence the copigment is probably an anthoxanthin.

(v) *Identification of flavones*

The flavone in the *yellow* flowers was identified by the following method. The flowers were boiled for half an hour with 1 % aqueous hydrochloric acid, filtered and the treatment repeated. The combined filtrates were cooled, and a saturated aqueous solution of lead acetate added until all the chloride had been precipitated. This was removed by filtration, and more lead acetate added to precipitate the lead salt of the flavone. If necessary the pH was adjusted by careful addition of ammonia. The yellow lead salt was filtered and triturated with hot 5 % sulphuric acid. After removal of lead sulphate the acidity was increased and the glucoside hydrolysed by boiling for 2–3 hr. The solution was filtered while hot, and on cooling deposited a brown amorphous solid. A further quantity of this crude flavone was obtained by saturating with sodium chloride. The crude product after drying was exhaustively extracted with ether (Soxhlet), the extract evaporated to dryness, and the yellow residue acetylated with acetic anhydride containing a few drops of pyridine. On pouring the resultant liquid into water a brown solid was precipitated, and this was extracted with hot methyl alcohol, leaving a sparingly soluble white solid which constituted the bulk of the crude

acetyl derivative. This was crystallized once from aqueous acetic acid and three times from acetone-ethyl alcohol and was obtained as long colourless needles having a melting-point 227–228° C. Luteolin tetra-acetate has a melting-point of 225–227° C. (Herzig, 1896).

On analysis the acetyl derivative gave

$$C = 60.95\ \%,\quad H = 3.9\ \%,\quad CH_3CO = 38.2\ \%.$$

$C_{15}H_6O_2(OOC.CH_3)_4$ requires

$$C = 60.8\ \%,\quad H = 4.0\ \%,\quad CH_3CO = 37.9\ \%.$$

The pigment is therefore a tetra-hydroxy-flavone glycoside, and although mixed melting-points have not yet been carried out, the evidence strongly suggests that the flavone is luteolin.

The flavone in the *white* flowers was purified by a somewhat different method. The flowers were extracted first with ethyl alcohol and pressed out. They were then boiled in water with a drop of acid and the solution filtered while hot. The residue was boiled up and filtered a further three times, and the filtrates combined. The original alcoholic extract, which contained much chlorophyll, was distilled off and the residue boiled with water. The solution so obtained was filtered and added to the aqueous solution.

The combined aqueous extracts were concentrated, saturated with sodium chloride and extracted five times with ethyl acetate. This was distilled off and the residue boiled three times with water. The extracts were filtered while hot and hydrolysed by boiling with an equal volume of concentrated hydrochloric acid. The orange-yellow precipitate obtained was filtered off and purified by dissolving in warm 50 % aqueous ethyl alcohol. On cooling a yellowish, non-crystalline solid separated. This was dried and acetylated as with the yellow flavone. The acetyl derivative was purified by two or three crystallizations (with charcoal) from methyl alcohol. Colourless crystals were obtained having a M.P. 181–2° C. (Apigenin triacetate, M.P. 181° C.)

On analysis the acetyl derivative gave
$C = 63.7\ \%,\quad H = 4.1\ \%,\quad CH_3CO = 26.6\ \%$ (mean of two determinations)
Apigenin triacetate, $C_{15}H_7O_2(OOC.CH_3)_3$ requires

$$C = 63.6\ \%,\quad H = 4.1\ \%,\quad CH_3CO = 32.6\ \%.$$

The melting-point and the carbon and hydrogen content agree remarkably well with those of apigenin triacetate, but for the present the identity of this anthoxanthin remains in doubt because of the serious discrepancy in the acetyl determination.

3. Results

(i) *Qualitative differences in anthocyanins* (M^d–M–m; P^d–P–p)

Four main types of anthocyanin occur in the garden *Verbena*, as shown in Table 1.

Table 1. *The principal anthocyanins occurring in* Verbena
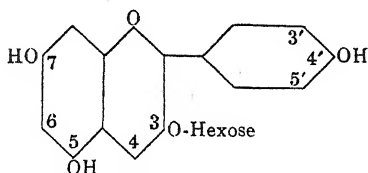
Pelargonidin 3-monoside
(scarlet)
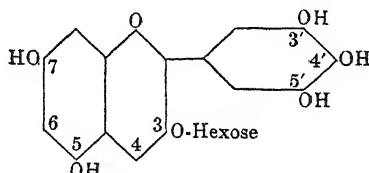
Delphinidin 3-monoside
(maroon)
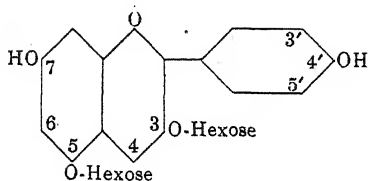
Pelargonidin 3 : 5-dimonoside
(scarlet-magenta)
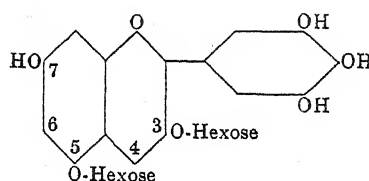
Delphinidin 3:5-dimonoside
(purple)

Reference to the genetical results given earlier (Beale, 1940) will show that there are two genetical types of scarlet, the first differing from purple by two dominant allelomorphs and the second by two recessives. Both dominant and recessive scarlets are however pigmented by precisely the same anthocyanin—pelargonidin 3-monoside. Similarly, of the two kinds of maroon, one differing from purple by a single dominant factor and the other by a single recessive, both contain the same anthocyanin— delphinidin 3-monoside; and the dominant and recessive scarlet-magentas each contain pelargonidin 3 : 5-dimonoside.

According to the genetical scheme put forward, there are two series of three multiple allelomorphs: (1) M^d–M–m, corresponding to the characters dominant 3-monoside, 3 : 5-dimonoside, recessive 3-monoside and (2) P^d–P–p, corresponding to the characters dominant pelargonidin, delphinidin, recessive pelargonidin. This scheme has been confirmed by additional data obtained recently. There are, however, further complications, e.g. sometimes the dominance is definitely intermediate and a

mixture of pelargonidin and delphinidin in approximately equal quantities is obtained (see Beale, 1940, Table 5).

In addition to delphinidin and pelargonidin, anthocyanins based on cyanidin occur in some strains. The cyanidin derivatives have so far never been found pure, but always mixed with either pelargonidin or delphinidin derivatives. The inheritance of these types has not been fully worked out, but on crossing a plant containing mixed pelargonidin and cyanidin derivatives with the dominant scarlet (pelargonidin 3-mono-side), the F_1 contained pure pelargonidin 3-monoside and in the F_2 the proportion of plants containing cyanidin + pelargonidin to those containing pure pelargonidin derivatives was 7 : 115. This indicates that the type containing cyanidin + pelargonidin derivatives (the variety "Crown Prince") differs from the dominant scarlet by one or more recessive factors, but the relationship between these and the factors controlling the pelargonidin-delphinidin difference is unknown.

(ii) *Variation in acylation of anthocyanins and*
pH *of cell sap* (**U–u**)

Purple, maroon, scarlet-magenta and scarlet flowers all contain acylated anthocyanins. Plum-coloured flowers (see *J. Genet.* **40**, Pl. XIII) are pigmented by non-acylated pelargonidin 3 : 5-dimonoside. The difference between the anthocyanins in plum and in scarlet-magenta flowers is therefore one of acylation. There are, however, two other associated differences: (1) In the cells of scarlet-magenta flowers the red pigment is distributed uniformly in the sap, while in the plum flowers there is a much darker, sharply delimited region in the middle of the cell where the anthocyanin is apparently more densely aggregated. Cells containing the dense structure occur chiefly towards the centre of the flower and are associated with the darker and bluer colour of this part of plum flowers. (2) The expressed sap of plum flowers is about 0·4 *p*H units more alkaline than that of scarlet-magenta flowers.

The genetical constitution of plum is **P^{d}M u** (or **p M u**), **M** and **u** being completely linked. Instead of **M** and **u** one might equally well write a single factor (say **M^u**) in the **M^d**, **M**, **m** series; but for reasons given previously (Beale, 1940) it has been thought preferable to postulate two factors, one controlling the difference between dimonoside and monoside, the other the difference between acylated and non-acylated anthocyanin (and associated differences). Plum contains the two factors in the repulsed condition, i.e. it is distinguished from scarlet-magenta by two effects, one dominant and one recessive.

An attempt has been made to determine the interaction of plum with other genes. The type plum-purple **P M u** is very similar in appearance to ordinary purple, though possibly a little bluer; the pigment from plum-purple is, as expected, non-acylated delphinidin 3 : 5-dimonoside. All efforts to get plum on to a scarlet or maroon background have failed, presumably owing to the tight linkage between **M** and **u**. Non-acylated 3-monoside types have therefore not been obtained.

(iii) *Copigment and flavone differences* (**D–d**; **A–a**; **Ye–ye**)

The incompletely recessive factor "dilute" (**d**) has a diluting and at the same time a blueing effect on the flower colour (see *J. Genet.* **40**, Pl. XIII). The blueing effect is caused by an increase in the degree of copigmentation; normally (i.e. in **DD** forms) there is comparatively little copigment present.

White and yellow flowers both contain flavone, not plastid pigments. The exact chemical difference between the two types is not known, but yellow flowers contain luteolin and white flowers another, unknown, flavone. The genetical difference between white and yellow is not a simple one, since in an F_2 derived from a hybrid between the two types a range of colours between the two parents is obtained. In F_1, however, yellow is completely recessive.

4. Discussion

When a comparison is made between the genetic relations of the various pigment types in *Verbena* and in other plants similarly investigated, it is apparent that *Verbena* is decidedly exceptional. Thus, in the great majority of plants (see Scott-Moncrieff, 1936) anthocyanins with more hydroxyl groups on the 2-phenyl ring are dominant to those with less (e.g. delphinidin is dominant to pelargonidin); in *Verbena*, however, delphinidin derivatives may be either dominant or recessive to pelargonidin according to which strain of scarlet is used. Secondly, in other genera there is a clear-cut segregation of anthocyanidin types and mixtures of two or more in one plant occur only rarely; in *Verbena*, on the contrary, mixtures of pelargonidin with delphinidin derivatives, and of both with cyanidin derivatives, are known. Thirdly, clear-cut segregation of differing glycosidal types, of acylated and non-acylated anthocyanins and of qualitative flavone differences, such as occurs in *Verbena*, is elsewhere uncommon or unknown.

Some at least of these irregularities are evidently due to the interspecific origin of the garden strains. The unusual dominance relations will be first considered.

Since mutants are usually recessive to their wild-type allelomorphs, it is important, when considering the dominance of a particular type, to know whether it is controlled by a wild-type or a mutant gene. The apparent regularity in the dominance of one anthocyanin type over another may be due to the fact that the direction of mutation is much more frequently from delphinidin to cyanidin or pelargonidin than in the reverse direction, and not to any fundamental cause affecting directly the dominance of one type over another. According to this view one would not expect a delphinidin type to be dominant over pelargonidin when (1) the two pigments have been derived from different species or (2) mutation has been in the unusual direction of pelargonidin to delphinidin. *Verbena* clearly is an example of class (1), and the most reasonable explanation is that the dominant pelargonidin factor has come from a wild-type allelomorph of a species containing a pelargonidin derivative—most probably the scarlet *V. peruviana*—and that the recessive pelargonidin factor has arisen by mutation. The apparent allelomorphism of the dominant and recessive pelargonidin factors needs to be confirmed before its significance can be adjudged.

A search is being made for examples of class (2). So far only one has been investigated fully, viz. *Anagallis arvensis*, in which the direction of mutation is from pelargonidin to delphinidin and in which the blue-flowered form containing a delphinidin derivative is, as expected, recessive to the red-flowered form containing a pelargonidin derivative. A similar example, *Salvia splendens*, is being studied (Beale, unpublished).

The occurrence of mixtures of different anthocyanins in the same plant, which is another unusual feature of *Verbena*, is also related to the interspecific origin of the plant, for so-called "blending inheritance" is common in species hybrids. This may be due either to the presence of modifying factors or to incomplete dominance.

As regards inheritance of glycosidal types, only one other example of a single factor controlling the difference between a 3 : 5-dimonoside and a 3-monoside has been established. This is in the China Aster, *Callistemma chinensis* (Wit, 1937), where dimonoside is dominant to monoside. This kind of variation is probably not so rare as was at first supposed, for in several other species (e.g. *Dianthus barbatus*, *D. caryophyllus*, *Phlox Drummondii*) two glycoside types occur, though their genetic relations have not been worked out. In *Verbena*, the presence of both dominant and recessive factors changing dimonoside to monoside is probably due to the same sort of cause as the presence of dominant and recessive factors changing delphinidin into pelargonidin; M^d is considered to be derived

from *V. peruviana*, which contains a 3-monoside, **M** from one of the other species (*V. platensis* contains a 3 : 5-dimonoside, and *V. incisa* and *V. phlogiflora* are inferred to contain 3 : 5-dimonosides from their appearance) and **m** by mutation. Here again, test matings with the actual wild species are required.

One other example of a gene controlling acylation of an anthocyanin is known, viz. in *Papaver Rhoeas* (Scott-Moncrieff, 1936). There, as in *Verbena*, the non-acylated type is the mutant, and recessive; it is also associated with a marked increase in alkalinity of the cell sap. Whether the increase in pH is the cause of the removal of acylation, or vice versa, is unknown. The correlation between non-acylation of an anthocyanin and its state of aggregation is also unexplained.

The inverse correlation between the quantity of anthocyanin and of flavone, as shown by the various phases of the factor **D**, is in accordance with the situation in numerous other plants (e.g. *Lathyrus odoratus*, *Primula sinensis*, *Dahlia variabilis*) where reduction in concentration of anthocyanin is invariably associated with increase in anthoxanthin.

There are scarcely any data on the inheritance of flavone or flavonol differences for comparison with those obtained from the white and yellow Verbenas. Wheldale & Bassett (1914) reported that in *Antirrhinum majus* there is an ivory coloured form containing apigenin and a yellow form, differing from the ivory by a single recessive factor, containing luteolin. This is apparently similar to the situation in *Verbena*, but recent work by Price (unpublished) indicates that the yellow pigment in *Antirrhinum* is not a flavone at all, but a chalkone. Hence it must be noted that there are at present no fully authenticated examples of a gene controlling flavone or flavonol differences in plants, though such examples will assuredly be discovered in the future.

5. Summary

1. The following types of variation in the flower pigments of *Verbena* (garden hybrids) have been established:

(*a*) The anthocyanidins may be pelargonidin, delphinidin, or mixtures of these with each other or with cyanidin (i.e. all combinations except cyanidin alone).

(*b*) The anthocyanins may be 3-monosides or 3 : 5-dimonosides.

(*c*) The anthocyanins may or may not be acylated.

(*d*) The non-acylated types have a more alkaline cell-sap, and in them the pigment is more densely aggregated.

(*e*) The anthocyanins may be partially or completely inhibited; if the former, there is an increase in anthoxanthin copigmentation.

(*f*) The anthoxanthins (in flowers not containing anthocyanin) may be luteolin (in yellow flowers) or an undetermined flavone (in white flowers).

2. The inheritance of these variations is contrary to rule in other plants in the following respects:

(*a*) Pelargonidin derivatives are sometimes dominant, sometimes recessive to delphinidin.

(*b*) Monosides are sometimes dominant, sometimes recessive to dimonosides.

(*c*) Mixtures of anthocyanins occur, due to incomplete dominance or to modifying factors.

These irregularities are considered to be due to the interspecific origin of the garden *Verbena*. The other variations are not abnormal as regards dominance, direction of mutation, or gene-interaction, in so far as they can be compared with homologous differences in other plants.

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THE GENETIC CONTROL OF WING DEVELOPMENT IN *DROSOPHILA*

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(With Plates 2–5 and Fifteen Text-figures)

CONTENTS

I. THE PUPAL DEVELOPMENT OF THE NORMAL WING, WITH A NOTE ON THE DEVELOPMENT OF THE LEGS

INTRODUCTION

THE wings of insects have for some years past been favourite objects for experimental studies in morphogenesis, and in particular for investigations on the mode of action of genes during development. An essential preliminary for such studies is a thorough knowledge of the normal processes of development. In the last few years very complete descriptions of the pupal development of the wings have been given by Köhler (1932) and Behrends (1936) for *Ephestia kühniella*, by Schlüter (1933) for *Habro-*

bracon, by Kuntze (1936) for *Philosamia,* and by Hundertmark (1936) for *Tenebrio.* Important genetical and experimental-morphological investigations have been made in all these forms, but in none of them do we yet possess anything like the wealth of genetical material which is available in *Drosophila,* and it is clear that if this material is to be fully utilized, we shall require for that form also an account of development as careful as those which have just been mentioned. This is already in existence, as far as the larval stages are concerned, in the work of Auerbach (1936), who also provides some data on the pupal development; and some further remarks on normal wing development are to be found in Chen (1929) and in the papers of Goldschmidt (1935, 1937) which are mainly concerned with the development of mutants, while a general account of the pupal period has been given by Robertson (1936). In none of these accounts of pupal development, however, are the data sufficiently complete to enable one to evaluate the divergences from the normal which characterize the different mutant types. The purpose of this paper is to provide a normal table of wing development from the time when the blade of the wing becomes distinguishable to the adult condition. A preliminary account has already been published (Waddington, 1939).

MATERIAL AND METHODS

The greater part of the material was prepared during a stay, between January and April 1939, at the California Institute of Technology, Pasadena;[1] the remainder was collected in the Zoological Laboratory, Cambridge. In both places, the stock used was a wild race known as Oregon-R. Pupae were collected from the sides of the culture bottles and incubated until required at 25° C. The zero time, from which their age is measured, was taken as the time at which movement of the anterior spiracles was no longer possible. Fixation was usually by hot water, which, as Robertson noted, seems nearly as satisfactory as any more refined method, since most fixatives are unable to penetrate the chitinous sheaths in which the animal is enclosed. For certain points, however, it was necessary to use Carnoy and to tear the chitin so as to allow the fluid to reach the tissues.

The wings were investigated as whole mounts after staining in Delafield's haematoxylin, or as serial sections cut at $7\,\mu$.

The developmental stages

It has been known since the investigations of Snodgrass (1924) on *Rhagoletis* that in Diptera the formation of the hard chitinous sheath, in which the animal becomes immobilized, is not necessarily a sign of pupation in the true sense. Robertson (1936) confirmed this for *Drosophila*, and showed that the animal inside the puparium undergoes a complete prepupal instar before pupation. The latter process does not occur till $11\frac{1}{2}$–12 hr. after the cessation of movement, and is announced by the comparatively sudden eversion of the head. Following Robertson, the animal, during the instar which separates puparium formation from pupation, will be called a prepupa.

v. Buddenbrock (1930) and Köhler (1932) have suggested dividing the period between two moults into a series of phases, which are repeated in regular sequence throughout the life of the animal. Following a moult, there is a resting stage (*Beharrungsphase*); a withdrawal and cell-division phase (*Chitinablösungs- und Zellteilungsphase*); a stretching and folding phase (*Streckungs- und Faltungsphase*); and a chitin-formation phase (*Chitinbildungsphase*) which is succeeded by the next moult. This system can be fairly easily applied to the *Drosophila* wing during the period with which we are concerned, and provides a simple classification into stages. We can first separate the two instars as the prepupal (PP) and the pupal (P); and within each instar we can number the phases mentioned above as 1, 2, 3 and 4; minor subdivisions of these phases can be indicated by the letters a, b, c, etc. The phases arrived at in this way can be identified without reference to the actual age of the animal, which is a great convenience in studying the processes of development in mutant stocks. At the same time, the developmental stage is of course very closely correlated with age. In my material the great variation in developmental stage attained by flies of the same age, to which attention was drawn by Goldschmidt, was not found, although there is a certain amount of variation, which becomes greater towards the end of pupal life.

Stages PP 1 and PP 2 (resting). 0–4 hr.

As far as the wing bud is concerned, there is no true resting stage at the beginning of the "pupal" period. In fact the evagination of the wing from the imaginal disk may begin during the end of the last larval instar, and continues throughout puparium formation and for the next few hours. The details of the process are of considerable importance in

connexion with the mode of action of genes, and will therefore require rather full treatment.

Before eversion, the wing area is a thickened region on the ventral side of the dorsal mesothoracic imaginal bud. The two buds lie near the dorsal surface of the larva, with their blunt ends pointing posteriorly, while in the anterior they are drawn out into a stalk which connects them with the hypodermis. This stalk is extremely thin and, as Robertson points out, is difficult to find in sections; but it is fairly easy to see in whole mounts, and the connexion with the hypodermis is not broken before puparium formation. The eversion of the wing starts by a folding of the thickened area upwards, that is to say into the cavity of the bud. The wing region is thereby formed into a hollow sac (Pl. 2, fig. 1). This is directed posteriorly along the length of the bud, and it elongates in this direction for some time, during which period the thin dorsal wall of the imaginal bud degenerates and disappears, thus leaving the wing free.

The wing fold is at first broad, its upper, dorsal, surface being smoothly rounded, while the lower one is wrinkled parallel to the position of folding. At the lower, more posterior edge of the opening which leads into the fold, there is also a thickened region, which Auerbach has described as the marginal ridge. On the upper surface, the smooth wing area ends anteriorly in two deep transverse folds which meet at a fairly obtuse angle; farther anteriorly than this the epithelium of the bud is corrugated by a complex series of transverse folds. All these folds are difficult to show in photographs or drawings, since they lie at different focuses.

The eversion of the wing is not a simple folding of an already formed plate, since there are some other changes in shape involved. The primary phase may be likened to the protrusion of the human tongue; as the wing fold elongates, it narrows from side to side and becomes more nearly cylindrical. The analogy is, however, not exact, since it fails to take into account another change which is proceeding at the same time. The whole bud, as has been said, lies more or less in the long axis of the larva, and the eversion of the wing at first proceeds in this direction. Very soon, however, the wing blade is bent laterally, so that it eventually assumes a direction at right angles to its original position, running from the dorsal surface down towards the ventral, with its length lying in a plane transverse to the length of the whole animal (Text-fig. 1). This bending was noticed by Auerbach, who thought that it was probably due to a more rapid transverse growth of the original broad and shallow wing fold. This is not the case. The bending is really due to a rotation of the fold

about its base. The point is of some importance, since, if Auerbach's account was correct, the length of the final wing would appear as the breadth of the first wing fold, and vice versa. According to the present account, on the other hand, the axis of the adult wing is in the early wing fold parallel to the long axis of the larva. On the other hand, the fact that the early wing fold narrows and becomes more cylindrical as it elongates, shows that the relation of adult shapes to shapes of the fold will not be simple; in the earliest wing fold the future long axis has been telescoped up.

The reason for the confident statement that the bending is really a rotation can be found in a study of the earliest signs of the veins. The
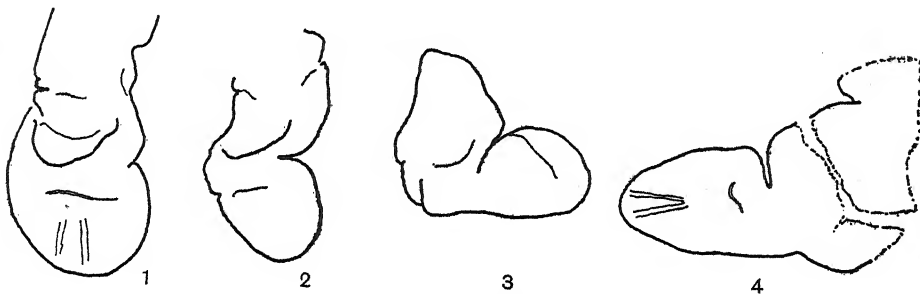
Text-fig. 1. The evagination of the wing in stages PP 1 and PP 2. The long axis of the larva runs along the length of the page, with the head at the top. The buds were drawn by transmitted light, as transparent objects. 1, ventral view of left bud, showing traces of veins; 2, dorsal view of right bud, showing early stage of bending of wing fold; 3, dorsal view of right bud, wing bent further; 4, dorsal view of left bud, wing fold elongated and showing traces of veins.

histological structure of these will be more fully described in the next stage, when they are better defined. But the very earliest signs of them can be seen in the partially everted wing, just before the time of the bending. At that time two fine pale lines may sometimes be made out running longitudinally; these are the traces of the two branches of the central lacuna which will be described in the prepupal wing. The fact that they run longitudinally before the bending shows that the final long axis is already longitudinally disposed at this stage. During the bending, their direction can be seen to alter in conformity with that of the wing as a whole, so that they always run along the length of the wing blade (Text-fig. 1; cf. Text-fig. 9).

At this stage the wing fold is usually still hollow, and the future veins are represented only by slightly wider spaces between the two surfaces. Corresponding to these wider spaces are rather feeble longitudinal folds on the outer, dorsal, surface (Text-fig. 2). We shall find

reason later, in the discussion of the action of some genes, to believe that these folds are not by any means so casual as one might think, but are on the contrary fairly rigidly determined and incapable of regulation in conformity to the size of the wing fold on which they lie.

The developmental processes which have just been summarized are rather rapid, and are completed in about 4 hours after puparium formation. There is considerable variation in developmental stage of different animals of the same age during this early period, and this is only to be expected, since the condition of the bud at puparium formation is sometimes more advanced and sometimes less so.
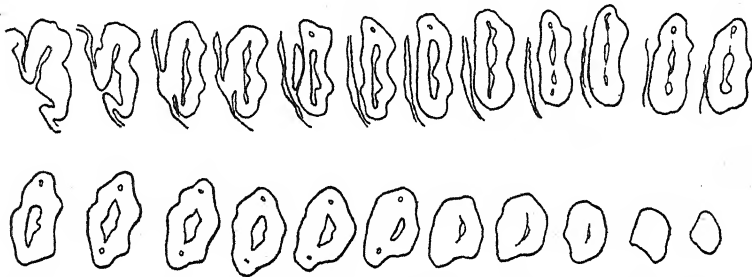
Text-fig. 2. Serial sections of the wing fold in stage PP 1, showing the feeble ridges on the dorsal surface and their connexion with the veins. Dorsal surface to right. The series runs from top left to the right, like letterpress in a book.

Stage PP 2 (withdrawal and cell division). 4–6 hr.

We may date the second stage of the prepupal period from the time when the wing has completely escaped from the peripodial membrane and is lying vertically along the side of the animal. The larval hypodermis may still be covering it at this time, but it is rapidly degenerating and soon disappears.

In whole mounts the wing appears as a solid, rather cylindrical block of tissue, about three times as long as it is broad (Pl. 2, fig. 2). In sections it can be seen that this solidity is not at all deceptive (Text-fig. 3 a). The two surfaces of the fold come together as they are invaginated into the interior of the imaginal bud, so that the inner cavity of the wing fold is almost obliterated. There are, however, certain lines along which the two surfaces do not come completely together, and thus hollow spaces are left. These constitute the first system of blood lacunae. They are disposed somewhat differently to the second (pupal) blood lacuna system from which the adult veins develop. There are, first, two marginal veins, along the anterior and posterior margins of the fold; that along the posterior margin, which has no analogue in the adult wing, is the thicker

of the two. One main lacuna runs down the centre of the wing, dividing at about the middle of the wing into two branches, both of which continue to the wing tip. These seem to correspond to the third and fourth longitudinal veins (medius and cubitus) of the adult. There is also a lacuna running in a curve just posterior to these two, and corresponding to the fifth vein; it joins the posterior marginal at a wide triangular-shaped junction. There is no trace of anything to correspond to the
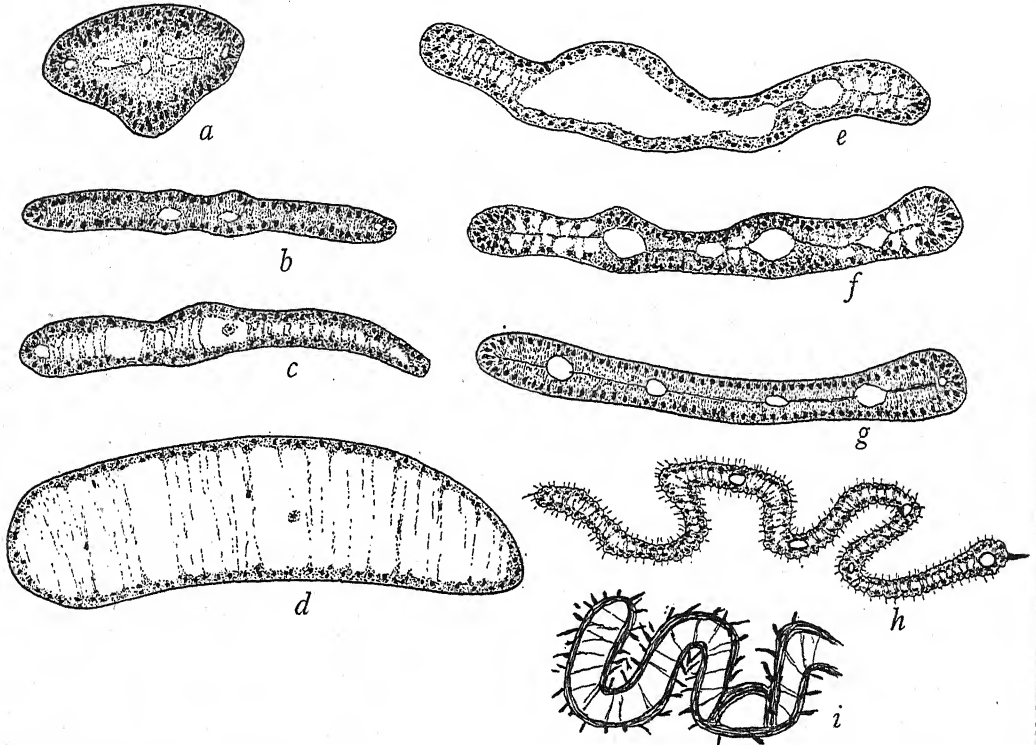
Text-fig. 3. Semi-diagrammatic sections of the wing. *a*, stage PP 2; *b*, stage PP 3; *c*, beginning stage PP 4; *d*, end stage PP 4; *e*, stage P 2*b*; *f*, end stage P 2*c*; *g*, stage P 2*d*; *h*, stage P 3; *i*, stage P 4 just before emergence.

second longitudinal (radius), nor can any cross-veins be made out (cf. Pl. 2, figs. 3–5).

The course of the blood lacunae is difficult to follow in whole mounts of this stage, owing to the thickness of the wing epithelia. These consist of high cylindrical cells, with the nuclei near the outer surface, although they are not arranged at any definite layer. The bases of the cells are drawn out as long protoplasmic cylinders. In some places a central membrane may be formed where the two surfaces are in contact, but it

only persists for a short time and has disappeared by the next stage. In this respect conditions are intermediate between those in *Tenebrio*, which has a central membrane, and *Habrobracon*, which has not.

Stage PP 3 (*stretching and folding*).   6–9 *hr.*

During the early part of this stage the wing begins to rotate again about its base, so as to bring its long axis once more nearly parallel to that of the animal as a whole. Histologically, the most important change is that the thick, solid wing blade becomes rapidly transformed into a thin plate of considerably larger area (Pl. 2, figs. 3, 4). The process seems to occur very quickly, since it is in the early part of this stage, more than in any other part of early development, that individual variation between animals of the same age is most noticeable. The thinning of the plate is mainly due to rearrangement of the cells. The epithelia, instead of being composed of high cylindrical cells, are formed of a single layer of quite flat cells, which are thin enough for the whole mounts to be fairly transparent (Text-fig. 3*b*). The blood lacuna system is therefore much easier to distinguish. The sudden thinning of the wing, which initiates the stage, causes a considerable expansion in area, and this continues throughout the remainder of the stage.

As regards the wing, this stage is predominantly one of stretching, and there is comparatively little sign of any folding of the wing surfaces. This folding is, however, very well seen in the legs.

It is during this stage that one can, for the first time, see the nerve and tracheole which extend into the wing. They enter near the anterior margin, where the central vein fuses with the anterior marginal, and the main branch runs down the central vein to about the point where it forks; a smaller branch runs forward a short distance along the anterior marginal vein. The two structures persist throughout the subsequent development, and presumably form a basis about which the veins L 1 and L 3 of the pupal wing are laid down. The other pupal veins, however, are not provided with either nerves or tracheoles, and must develop independently of them.

Stage PP 4 (*chitin formation*).   9–12 *hr.*

The stage of chitin formation is not sharply distinguished from that of stretching. The wing begins to secrete a chitinous sheath at about 6 hr. after puparium formation, but this sheath is at first not only extremely thin, but is also clearly extensible, since it grows as the wing expands. In fixed pupae it can fairly easily be removed from the wing,

as is necessary if proper staining is to be achieved. The chitin which is deposited in the present stage is thicker and more abundant, and becomes more difficult to remove without damage to the underlying epithelium. The expansion in area which was described for the last stage also continues throughout the present one, and even beyond. The distinction between the stages is, however, not entirely artificial, since the mechanism of the expansion is somewhat different in the two periods.

In whole mounts of this stage the wing is seen to be not only increasing in size, but also to become more transparent, while the system of blood lacunae becomes more difficult to make out (Pl. 2, figs. 5, 6). In sections it is seen that the cells of the wing surfaces are still increasing in surface area and decreasing in thickness (Text-fig. 3c). This does not apply to the wing as a whole. Indeed, it now begins an increase in thickness which, in the next stage, attains remarkable dimensions. This is achieved by the appearance of spaces between the basal ends of the cells. It is now impossible to discern any central membrane; the bases of the cells appear to be drawn out into long thin processes which stretch from one surface of the wing to the other, and the main system of blood lacunae can only be distinguished as rather larger and more continuous spaces extending through the spongy interior of the wing. A somewhat similar phenomenon was described by Hundertmark in *Tenebrio* and by Schlüter in *Habrobracon*, except that in the former case a middle membrane was always present.

At about the middle of this stage, at about 11 hr. after puparium formation, the wing has a very characteristic appearance (Pl. 2, fig. 6). Its central parts are still fairly flat, although somewhat swollen; the blood lacunae can be made out without much difficulty, particularly the two branches of the median lacuna. But at the edges, and in the region of the tip, the wing is difficult to stain completely in whole mounts, in which these peripheral portions usually have a somewhat washed out and fuzzy appearance. In sections it can be seen that the central part of the wing is considerably inflated, the two surfaces being only connected by processes in the manner described above, while the peripheral regions are still flat, with the two surfaces in contact. Moreover, at the wing tip and along the edges there are thick deposits of chitin closely adherent to the epithelia. It is probably a combination of the comparatively close texture of the interior and the thick cuticular covering which makes these regions less easy to stain than the central part.

The superficial appearance of a whole mount of this stage might be taken to suggest that the peripheral parts of the wing were degenerating

and were about to disappear. A process of this kind is known to occur in the peripheral regions of the wings of certain Lepidoptera (Sueffert, 1929). But it is extremely unlikely that any such event occurs in *Drosophila* at this stage. No trace of recently shed tissue can be found within the chitinous sheath, although the circulation in these regions must be exceedingly inefficient at removing debris. It should be remarked that the marginal degeneration postulated by Goldschmidt in certain mutant *Drosophila* wings is supposed to occur at a later stage (in P 2). It is discussed in a later section. Here it may be said that the only appearances during normal development which could possibly be interpreted as a degeneration are those which occur during the present stage and which have just been discussed.

Stage P 1 (resting).   12–18 hr.

This stage begins with the eversion of the head, the time from which the true pupal period is usually reckoned.

At the end of the previous stage, the wing is already a fairly thick sac, with thin walls which enclose a loose spongy interior (Pl. 2, figs. 7, 9). The growth in thickness of the wing continues probably until about 15 hr., that is to say somewhat after the eversion of the head. At this time the wing may be almost circular in cross-section, but it is almost completely empty of tissue (Text-fig. 3 d). The surface membranes are very thin and tightly stretched, lying closely apposed to the chitinous cuticle. Within the sac one can at first trace the elongated basal processes of the cells stretching across from one surface to the other. At the height of the expansion, however, it seems that most of these processes are broken. A few can usually be made out in the distal and proximal regions of the wing, but in the central region there is no sign of them. Their absence is, however, difficult to prove conclusively, since they may be so thin as to be almost invisible.

In those regions where they can be definitely seen, one often finds a cell whose base is drawn out to a point, as though attached to a fibre running across the wing, although no fibre may be visible. In the central regions of the wing, however, even these indications of possible fibres are lacking, and there seems little doubt that the connexion between the two surfaces is entirely broken. The process of inflation thus goes much farther in *Drosophila* than in *Tenebrio* or *Habrobracon*, where it is also a noticeable feature of this phase of development. Whether this is generally so in the Diptera has not been determined.

Stage P 2 (withdrawal and cell division). 18–45 *hr.*

During this phase the wing contracts again to a thin blade, which lies freely within the large sac of pupal cuticle. As the contraction occurs, the definitive blood sinuses appear, and from these the adult veins are directly formed. Since the veins are one of the most important features of the wing, from the point of view of an attack on the mode of action of genes during development, it is necessary to consider their formation in detail, and it therefore appears advisable to divide this stage into substages.

Substage P 2 a (Pl. 2, fig. 8). The first phase in the contraction of the wing from the inflated bag of the last stage occurs when the two wing surfaces begin to come together at the tip and around the margin. In these regions, at least at the tip, it is probable that even at the stage of fullest expansion some connecting fibres between the upper and lower surfaces have persisted. The contraction may well be initiated by the shortening of these fibres. But this can scarcely be all that is involved, since as the two surfaces come together a well-marked central membrane is formed. The appearances suggest that as the two surfaces approach the cells put out basal processes, and that when these meet they form a central membrane between them.

The stage P 2 a is considered to be complete when the two surfaces have made contact all round the margin of the wing, which then has the appearance of a thin-walled sac with a thick ridge round it, along the margin. This occurs at about 19 hr.

Substage P 2 b (Pl. 2, fig. 10, Text-fig. 3 e). In this stage the veins begin to appear. The first to be visible are two veins at the tip of the wing, where the contraction is taking place most rapidly. They correspond to L 3 and L 4 of the adult wing. Very shortly afterwards the contraction, which is progressing fast, particularly round the margins, begins to be noticeable towards the root of the wing, where it cuts off and delimits a large space from which the bases of the veins will eventually form. At the end of stage P 2 b, however, this space is still undivided; it is a cavity fairly sharply outlined by regions in which the two surfaces are in contact; immediately distal to it, in the centre of the wing, is an uncontracted region, while again at the tip of the wing, contact between the surfaces has been attained except in the region of the two veins L 3 and L 4 mentioned above. This condition is reached at about 20 hr.

Substage P 2 c (Pl. 2, figs. 11–13, Text-fig. 3 f). During this stage the contraction spreads from the distal and proximal regions, and also from

the margins inwards, and obliterates the central vesicle which has just been described. In the process the remaining veins appear. The final disappearance of the central vesicle takes place in the region of the posterior cross-vein, which can in fact be considered merely as a last vestige of it. The anterior cross-vein is at first indicated merely by the nearness of veins L 3 and L 4. The veins are at this time much wider than they are in later stages, and the anterior cross-vein appears at the place where L 3 and L 4 were in contact with one another, becoming a definite vein in its own right as these two become narrower and therefore would tend to lose contact with one another.

At the end of this process all the veins are present except for the radius, L 2. The intervein material is still spongy in texture, since there are fairly large spaces between the basal process of the cells. In the wing epithelia, cell division is proceeding more actively, and is particularly rapid immediately above and below the lacunae. The cells of the vein surfaces thus become crowded together, and assume a high columnar form, with rather deeply staining cytoplasm, whereas over the rest of the surface they are still flat and drawn out beneath in the processes which extend to the central membrane. This stage is reached at about 24 hr.

During the later part of this stage the radius (L 2) is formed. In the manner of its appearance it differs from all the other veins, since it is not formed directly as a space left when the upper and lower surfaces are coming together. On the contrary, the region between L 3 and the anterior margin is at first filled with spongy tissue, constituted by the basal processes of the cells as was described above. The radius begins to appear as an extension of the large cavity which has persisted at the base of the wing since stage P 2b. It appears that along the line of this vein the spaces between the basal processes enlarge and fuse together to form a continuous channel, while above and below this the epithelium thickens to form the typical vein surface. It is likely that a similar process also starts at the point where the vein will meet the marginal vein, and progresses basipetally from there; but the greater part of the vein is certainly formed by extension from the base towards the tip. The process appears to be somewhat irregular, both in the exact stage at which it begins and in the speed with which it is carried out, since in different specimens showing half-formed radii, the width of the other veins is rather variable. The process is, however, usually complete by about 28 hr., and at that time all the veins of the adult are laid down.

The formation of the radius by a means different to that of the other veins does not appear to have been described in any other form.

A word should be said as to the formation of the marginal vein. It is first necessary to point out that no vein appears in the posterior margin at this time; the large sinus which was found here in the prepupal wing has no representative in the pupa. Along the anterior margin there is, from the very beginning of the contraction, some sign of a vein in the sense that the tissue there is markedly thickened. But there is at first no lumen, and this only appears gradually, at first as a narrow discontinuous tube which later becomes continuous. It remains, however, much narrower than the other veins until the later part of the next stage.

Substage P 2*d* (Pl. 2, figs. 14, 15, Text-fig. 3*g*). During this stage the veins become narrower, and at the same time the epithelia become more solid. The stage is probably, in fact, one of cell division, which seems to spread from the vein surfaces over the entire wing blade. The spongy texture of the intervein regions disappears, and the small spaces between the basal processes are lost. The whole epithelium acquires much the same appearance as that over the vein surfaces, except that in the intervein regions the cells are higher. The central membrane persists, and is in fact very obvious. The wing, which had contracted somewhat in area during the foregoing stages, expands again slightly.

The stage is a long one; it closes at about 45 hr., by which time the wing is a flat plate, complete in nearly every detail. The hairs, the first traces of which can be made out at about 30 hr., are by now present over the whole surface, but are still merely thin protoplasmic processes. This is the last stage before the adult at which the wing can be easily investigated, and it may be called the definitive wing stage.

Stage P 3 (*stretching and folding*). 45–60 *hr*. (Pl. 2, fig. 16; Text-fig. 3*h*)

At the end of the definitive wing stage the epithelia of the two wing surfaces are quite dense, there being no spaces between the bases of the cells, which remain plump and well filled with cytoplasm right down to the central membrane. After about 45 hr. the wing begins once more to expand, much as it did in stage PP 3. Spaces appear near the bases of the cells, and gradually enlarge. Immediately the spaces become noticeable, the central membrane disappears, and the central region of the wing is occupied by protoplasmic processes extending from one wing surface to the other.

A similar disappearance of the central membrane during the corresponding stage was noted by Schlüter in *Habrobracon*. In that form it was accompanied by a migration of cells, or at least of nuclei, from the surface epithelia into the interior of the wing. This migration does not

occur to any great extent in *Drosophila* at this stage, though there may
be a few nuclei which do not lie at the surface. The cells of the epithelia,
however, become interdigitated with one another, so that the surface
is no longer smooth but is made up of alternately protruding and re-
treating cells.

The expansion of the wing at this stage is clearly dependent on the
swelling of the cells; it appears that the rate of cell division is not
markedly increased, and it may in fact have ceased entirely. The swelling
may plausibly be attributed to an imbibition of water. If pupal wings are
dissected out in tap water or distilled water at any stage during pupal
life, except perhaps during the stage of maximum expansion in stage
PP 4, they very rapidly expand in area; in physiological salt solution
this does not occur. This is certainly an osmotic swelling, and the ex-
pansion which normally occurs during development is probably of the
same kind.

Since the wings are expanding within the limited space available
inside the pupal chitin, they become creased and folded. The folds follow
a definite pattern, which, however, has not been made out in detail.

Stage P 4 (chitin formation). 60–96 *hr.* (Text-fig. 3 *i*)

At some point during the folding and expansion, the deposition of
the adult chitin begins. The exact time at which this first becomes
noticeable has not been ascertained, but it is in the region of 60 hr.

In the pupa just before emergence, the wings are heavily chitinized,
and the greater part of the cytoplasmic substance of the cells has dis-
appeared. The two surfaces are connected by rather coarse fibres, which
run from side to side with no sign of a central membrane. The chitin is
equally thick on the dorsal and ventral surfaces, but is considerably
thickened round the veins.

After emergence the wing is unfolded and flattened partly by move-
ments of the legs and partly, it appears, by the internal pressure of the
body fluids. The latter presumably withdraw into the body as the wing
dries out.

CHANGES IN THE SHAPE OF THE WING DURING THE PUPAL PERIOD

It is during the pupal period that the wing assumes its final adult
shape. The changes involved are somewhat complex. During the early
part of the period, the thick inflated wing becomes a flat plate. The
contraction is not only in thickness, but also in area. The magnitude of
the contraction can be best estimated by reference to the chitinous pupal

sheath, which attains its final form during the period when the wing is most fully inflated. By the end of stage P 2c, the wing is already fairly thin and flat, but it lies loosely within the pupal sheath. When both the wing and the sheath are flattened out under a cover-slip, the relative areas are about 100 : 70.

It is clear that by stage P 3 the wing has already started to expand in area again, and it is probable that the change from a contraction to an expansion occurs some time during the later phases of stage P 2. But it is difficult to determine this point at all exactly, nor is it, for any purposes at present in sight, very important to do so. More interest attaches to the question of relative changes in shape of different regions of the wing. If one compares a wing of stage P 2c with one from the end of stage P 2 or the very beginning of stage P 3 (Text-fig. 4a), it will be seen that

Text-fig. 4. Changes in shape of the pupal wing. a, wing of stage P 2c, in full lines, super-posed on one of the end of stage P 2d (dotted lines), note the relative contraction of the proximal part during this interval; b, wing of late stage P 2d (full lines) super-posed on an adult wing reduced to the same size (dotted lines), note contraction of basal part of wing.

the most important change which has taken place is a relatively greater contraction of the proximal region. In fact, it seems that the proximal region continues to contract in absolute size even after the time at which the distal regions begin to expand; one of the most noticeable effects of this is that the posterior cross-vein becomes farther removed from the margin. The contraction of the proximal region is certainly connected with the narrowing of the veins, which continues right through stage P 2 and even beyond. Since the proximal region has a much higher pro-portion of vein material than more distal parts, it is to be expected that it will contract for a longer time and to a greater extent. Its relative contraction in fact continues throughout the expansion period of P 3 and P 4, since if early P 3 wings are compared with adult ones (Text-fig. 4b), the most noticeable difference in their properties is the greater relative breadth of the proximal region of the former.

During the contraction the outline of the wing undergoes certain rather subtle changes which bring it into the adult shape. There is a certain pointing of the tip; and as the proximal region contracts, the

region posterior to L 5 becomes well rounded, and L 5 itself is bent into a sharper and more tense-looking curve. These changes are, as we shall see, probably dependent on the same forces as those which are responsible for the distortion of the wing in mutant types such as dumpy.

<center>THE DEVELOPMENT OF THE LEGS</center>

The development of the legs in the larval period, and their eversion from the peripodial sacs in the early prepupa, have been fully described by Auerbach, and nothing need be added to her account. After puparium formation the legs undergo changes which illustrate very well the succession of two instars, and exhibit particularly clearly the division of the prepupal instar into phases of resting; withdrawal and growth; stretching and folding; and chitin formation.

The leg becomes free from the peripodial sac in the withdrawal stage PP 2. At this time the segmentation is very clear in whole mounts (Pl. 3, fig. 13). In sections the leg is seen to be composed of a rather thick epithelium, with the nuclei lying near the surface. A vein and a nerve run down to the tip of each leg (Text-fig. 5 a).

In the following stage, PP 3, the legs undergo great extension. At the same time they become highly folded, showing this feature much more markedly than do the wings (Pl. 3, fig. 14; Text-fig. 5 b). The numerous creases and folds obscure the segmentation, which can no longer be made out. In the following stages, PP 4 and P 1, the folded leg is expanded and blown out in a way similar to that described for the wings. The legs are at this time very difficult to prepare in whole mounts, since the surface is closely invested by the chitinous covering, which is difficult to remove without injury, and which prevents proper staining. In sections of the fully inflated stage, the epithelia are very thin, and the cells only just retain contact with one another. No sign of the segmentation can be made out (Pl. 3, figs. 15, 16; Text-figs. 5 c, 5 d).

In stage P 2 the legs begin to contract again from their inflated form. The contraction starts at the distal end and progresses proximally, and as it proceeds the epithelium becomes thicker (Text-fig. 5 e). It appears that a basement membrane is formed, but this cannot be made out satisfactorily. The joints between the segments appear as slight foldings (Pl. 3, fig. 17), which gradually cut more and more deeply into the shaft of the leg. The definitive leg stage, at which all the adult structures can be recognized, is reached at about the same time as the definitive wing stage (Pl. 3, fig. 18). Subsequent to this the legs remain more or less unchanged, except for the deposition of the chitin of the adult instar.

There is no folding comparable to that seen in the wing in the stages P 3 and P 4.
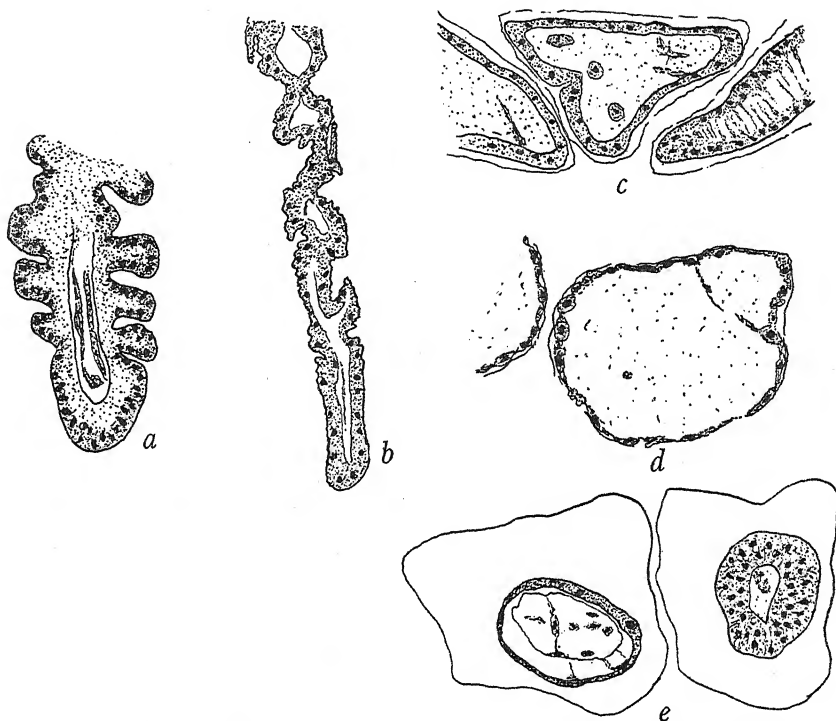
Text-fig. 5. Semi-diagrammatic sections of legs. *a*, stage PP 2, longitudinal section of tarsal region, showing the folds which correspond to the tarsal joints; *b*, longitudinal section of the tarsal part during stage PP 3; *c*, transverse section of tarsus of third leg near the wing tip at the beginning of stage PP 4; *d*, transverse section of tarsus of third leg near the wing tip during stage P 1, at the time of maximum inflation; *e*, transverse sections of tarsi of second and third legs near the wing tip during stage P 2*b*; the section cuts the distal joint of the second leg (on the right), and a more proximal joint of the third leg.

DISCUSSION

This section of the paper is intended only as a straightforward description of the development of the wings, and the many interesting bearings of the data which have been presented on questions of genic action are reserved for later discussion.

Comparing the wing development in *Drosophila* with that of other insects, perhaps the most remarkable features which it shows are the extreme inflation of the wing at the end of the prepupal period, and the transitory nature of the central membrane. Of the other insects whose wing development has been fully described, *Tenebrio* is the one which

most nearly approaches *Drosophila* in the degree of inflation, but even it is far behind. Moreover, *Tenebrio* possesses a central membrane throughout the prepupal period, and this persists during the expansion, although the cells of the two surfaces may pull apart, so that the single central membrane breaks down into two basement membranes which are not quite in contact with one another. The complete disappearance of the central membrane has been noted in *Tenebrio*, but only in the region of the posterior edge of the hind-wing, towards the end of the chitin-formation stage of the pupa (stage P 4). In *Habrobracon*, however, the central membrane is more transitory, and conditions in this respect approach those in *Drosophila*.

It is perhaps worth drawing attention to the fact that the adult veins develop directly out of blood lacunae, and that these in their turn are not dependent on the presence of tracheae or tracheoles, but are anatomical entities in their own right.

Summary

Development after puparium formation comprises the prepupal and pupal instars. Each of these can be divided into four phases: resting, withdrawal and cell division, stretching and folding, and chitin formation. Applying this scheme to wing development, the events can be summarized as follows:

First prepupal stage PP 1, *0–4 hr.* The wing is everted by being pushed dorsally through the peripodial sac. It is thick and fairly narrow and hollow, the prepupal veins being represented by ridges on the dorsal surface which are reflected by wider spaces within the cavity of the wing. At first its long axis is in the long axis of the larva, but it soon turns to lie at right angles to this, in the transverse plane of the larva.

Second prepupal stage, PP 2, *4–6 hr.* The wing becomes somewhat thinner and flatter. The prepupal venation becomes easily visible; it consists of a main longitudinal vein which branches at about the middle of the wing, a posterior vein following the course of the adult L 5, and veins along the anterior and posterior margins.

Third prepupal stage, PP 3, *6–9 hr.* The thinning of the wing continues rapidly, and the wing rotates again so as to lie more or less along the long axis of the larva. There is comparatively little folding of the wing, but this is marked in the legs. The vein and tracheole have entered the wing by this stage.

Fourth prepupal stage, PP 4, *9–12 hr.* The wing expands in thickness by an inflation which forces the two surfaces apart. The bases of the

epithelial cells are drawn out in long thin processes, and the venation becomes obscured.

First pupal stage, P 1, *12–18 hr.* The inflation of the wing persists, or even increases. Most of the basal processes probably break, leaving the two wing surfaces unconnected.

Second pupal stage, P 2, *18–45 hr.* The wing contracts again to a flat plate. Owing to the importance of the sequence of events, the stage may be subdivided as follows:

P 2*a*, *18–19 hr.* The wing contracts along the margin, becoming a hollow sac with a thickened seam.

P 2*b*, *19–20 hr.* Contraction spreads from the distal end, and tips of two longitudinal veins (L 3 and L 4) make their appearance. Contraction also starts proximally, leaving an uncontracted "central vesicle" in the middle of the wing. As the wing surfaces come in contact, the cells of the epithelia put out basal processes which unite to form a central membrane. The cavities between the basal processes give the contracted parts of the wing a spongy appearance.

P 2*c*, *20–28 hr.* The rest of the venation appears. L 3, L 4 and L 5 appear as persisting cavities as the two surfaces come together. L 2 is formed by the coalescence of cavities, so that it seems to develop out of a spongy region. The anterior cross-vein is at this stage only a place where L 3 and L 4 unite; it gradually acquires a separate existence as these two become narrower. The posterior cross-vein is the last remnant of the central vesicle. The anterior marginal is formed gradually in the thickened anterior margin.

P 2*d*, *28–45 hr.* The veins become narrower, and the epithelia more compact, so that the spaces between the basal processes disappear, and the spongy appearance is lost.

Third pupal stage, P 3, *45–60 hr.* The wing expands by an expansion of the cells, and it also becomes folded. The central membrane disappears, the epithelia being connected by comparatively few coarse fibres.

Fourth pupal stage, P 4, *60 hr. to emergence.* Chitin is deposited, and the living material degenerates and disappears.

The most remarkable feature of this development, compared with that in other insects, is probably the great exaggeration of the inflation of the wing at about the time of pupation.

II. DEVELOPMENT OF SOME MUTANT TYPES

INTRODUCTION

There are few objects as well suited for the study of the genetic control of morphological development as are the wings of *Drosophila*. Not only is their structure rather simple when compared to that of most animal organs, but we possess a very large number of genes which affect it. With such a wealth of material one may hope, first, to analyse the morphogenetic process into its constituent phases, and secondly to determine the ways in which these developmental processes can be modified by gene substitutions. Ultimately, one must aim at determining the chemical mechanisms by which the gene effects are produced. But in asking this last question, one is raising the general problem of the chemical basis of morphology, a problem which lies at the root of the whole phenomenon of animal form, and finally of animal function also. The solutions of it which can at present be offered are, as is well known, of an extremely tentative and general kind, and one can hardly expect to be able to resolve at one blow the two problems of the chemical nature of morphogenesis and of gene action. The data which are presented in this paper are offered merely as an approach to these two questions, which, although it does not yet carry us all the way to a solution, nevertheless seems to hold out some prospect of advance.

We shall find that the three most important elements in the wing, from the developmental point of view, are the epithelial sac, the veins, and the margin. It might at first sight seem sensible to study successively the genes which affect these different elements; but in fact this would be a misplaced simplification. The three are not separate and independent entities, but are bound together in a continual interplay of causal relations. It has therefore seemed better to approach the matter extensively rather than intensively, and to investigate a number of genes. This means, however, that the potential field of study is very large. One has, awaiting investigation, not only the numerous individual genes, but also their combinations, a full study of which will occupy a very considerable time. The present account, therefore, although it deals with some matters in fair detail, is to be taken as in some measure an interim report, since the conclusions which are drawn in it may have later to be modified in accordance with investigations which can already be envisaged, and are, in part, already in progress.

Nearly all the genes studied were in *Drosophila melanogaster*, and are kept in the stocks at Pasadena. The histological treatment was as re-

corded in the previous part of this paper. A preliminary notice of some of the results was published in Waddington (1939).

THE DUMPY PHENOMENON

One of the most characteristic mutant wing shapes is that produced by the dumpy allelomorphs; a similar effect is caused by spade and possibly by other mutants which have not been examined in this study. Dumpy wings are shorter than normal, and the distal end is characteristically curved inwards to give a concave margin. The effect can be found in a series of grades, the weakest of which is merely a slight flattening of the tip of the wing (Text-fig. 6 a).

Goldschmidt (1937) and Auerbach have both studied the development of dumpy wings, and have pointed out that it is quite normal in early stages, the characteristic shape only developing fairly late in pupal life. In more precise terms, the shape is normal up to the inflated bag stage, PP 4 and P 1, and the dumpy effect becomes noticeable during the contraction stages of P 2. Auerbach offered no explanation of the gradual alteration to the dumpy shape, but Goldschmidt suggested that there is a degeneration of subepidermal tissue at the wing tip, which leads to a collapse of the margin there. At one place he speaks of a degeneration affecting the mesoderm only. The data on the histology of wing development, which were not available to Goldschmidt, make it clear that this explanation is not satisfactory as it stands, since there is in fact no subepidermal or mesodermal tissue which could degenerate in this way. Further, a glance at a dumpy wing shows that the effect is by no means a localized one; not only is the wing margin bent inwards at the tip, but the proximal part of the wing is actually wider than normal, and the whole set of veins is distorted.

If one bears in mind the developmental processes which are known to be proceeding at the time when the dumpy shape becomes apparent, the explanation of the dumpy phenomenon immediately presents itself. At that time the wing is contracting from an inflated sac to a flat plate of considerably smaller area, and the veins are appearing as lines of more coherent epithelium lining hollow canals. The dumpy effect is due simply to a disproportion between the forces due to the contracting wing epithelia and those due to the veins, which as they develop gradually give support to the wing blade. This immediately accounts for the general and unlocalized changes characteristic of dumpy, such as the widening of the basal part of the wing. Moreover, it enables one to see the connexion between dumpy and another type of wing, that caused by Blade
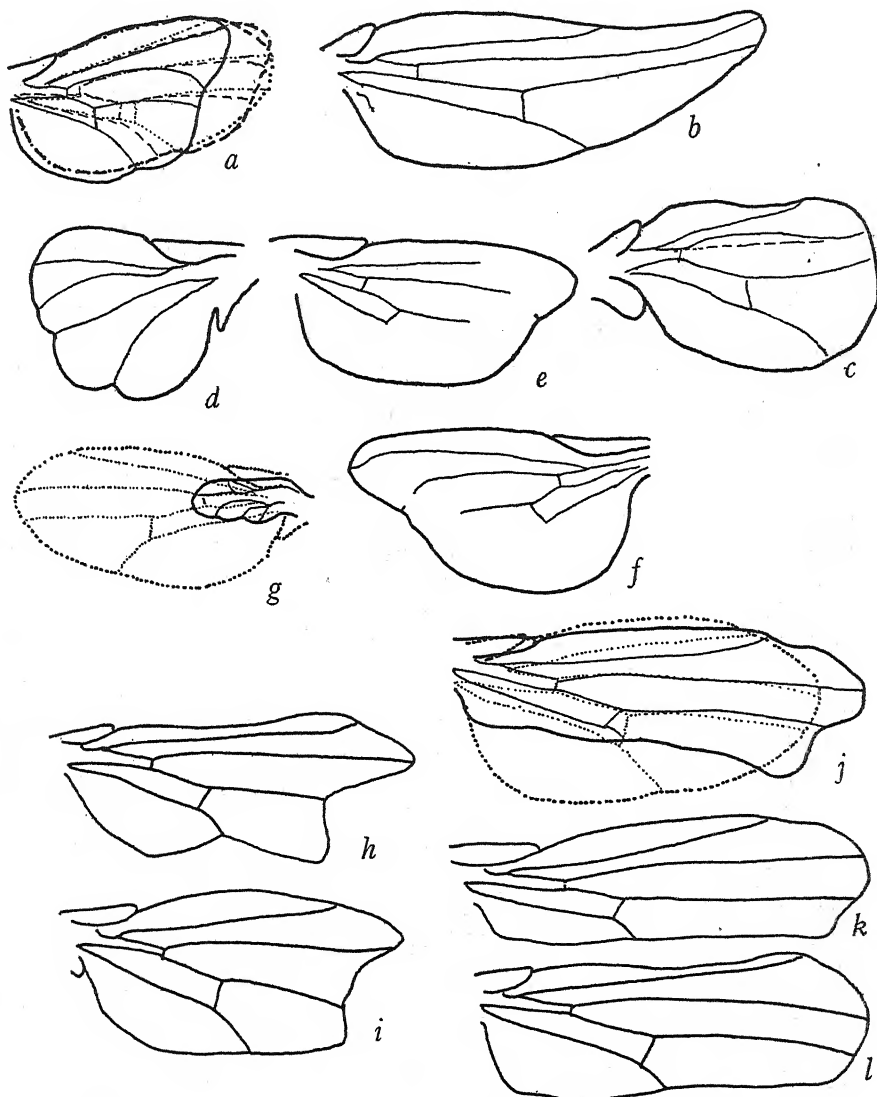
Text-fig. 6. The dumpy phenomenon. *a*, various grades of dumpy (dotted = normal wing reduced to same size, dashed = weak dumpy, full line = strong one); *b*, Blade, usual type; *c*, Blade, dumpy type, from same fly as fig. *b*; *d*, dumpy-cut-6; *e*, dumpy-veinlet, note the sharp angle between the two parts of L 3; *f*, dumpy-veinlet, L 3 curved so as to meet its tip smoothly; *g*, vestigial (full line) superposed on wild-type (dotted) reduced to equivalent size, note the spreading of *vg* veins; *h*, vestigial-notched, wing of elongated type, note acute angle between L 2 and L 5; *i*, vestigial-notched, wing of more rounded shape, angle between L 2 and L 5 normal; *j*, an elongated Beadex-C wing (full lines) superposed on equivalent normal (dotted) to show squeezing together of longitudinal veins; *k*, Beadex-C; *l*, Lyra, note squeezing together of longitudinal veins.

in *D. pseudo-obscura*, which at first sight seems completely opposite to dumpy, but which must be in some way connected with it, since the Blade factor occasionally produces dumpy effects (Text-fig. 6 c). In Blade the wing is long and drawn out towards the tip, but like dumpy has a complete marginal vein (Text-fig. 6 b). The effect here could be due to an increased tension of the contracting wing sac in the transverse plane, whereas in dumpy the tension seems to be abnormally great in the longitudinal plane of the wing. The development of Blade fits in with such a supposition to the extent that here again the wing is normal in shape until the contraction stages begin (Pl. 4, figs. 5–8).

As has been mentioned, flies carrying Blade occasionally have dumpy wings. This seems to be due to slight environmental effects, since flies have been seen which had one wing Blade and the other dumpy in type. It is probable therefore that as the contraction begins, the decision as to whether the tension shall develop in the longitudinal or the transverse planes turns on very minor alterations in conditions. One can easily understand that once the wing has begun to elongate, as in Blade, or to shorten, as in dumpy, the tendency to go on changing in the same way would increase cumulatively.

There are no data which would allow one to discover the crucial conditions which decide between the Blade and dumpy results of increased epithelial tension. One might suggest that the elasticity of the anterior marginal thickening, which later becomes vein L 1, was the decisive factor, since there is evidence that the contraction of the sac is checked by the developing veins. This can be seen in several ways. For instance, in the compound of dumpy with cut, the cut gene causes the partial disappearance of the anterior margin, and it is found that the wing becomes even more rounded than is normal in dumpy, and that the rounding of the tip of the wing begins proximal to the distal end of L 2, exactly as though the contracting sac had been able more easily to bend the weakened margin (Text-fig. 6 d). Again, many dumpy wings are ill formed, and appear crumpled. On examination it will be found that in these wings the veins are too long for the membrane between them; it is as if the contraction had acted a little later than usually, and was unable to cause the necessary shortening of the veins.

This raises another point. We have spoken of the dumpy effect as produced by the contraction of the epithelium. But a general distortion of the wing might also be brought about if the veins had a specific tendency to assume a certain pattern, and in doing so dragged the epithelia after them. The fact that the tendency in dumpy is for the

veins to be too long, and the epithelia too small in area, shows, however, that the contracted shape of dumpy cannot be due to the shortening of the veins. Comparable data for the Blade type, in which one would expect the tendency to be for the veins to be too short, are not to hand, since Blade-type wings are usually well formed.

The only other agent which one might imagine could produce a generalized effect such as the dumpy wing would be a specific distortion of the margin, reacting on the rest of the wing surface. The possibility of this can be to some extent tested by making up the compound of dumpy and veinlet. The latter gene causes the disappearance of the distal ends of the longitudinal veins, so that the margin becomes connected with the rest of the wing only through the thin epithelia instead of through the fairly thick veins as well. In this compound the dumpy shape is perfectly developed, and it seems difficult to believe that a contraction of the margin, by squeezing up the epithelium, has caused the distortions of the vein system; the epithelium may reasonably be supposed to possess considerable tensile strength but hardly any resistance to compressive stresses (Text-figs. 6c, 6b).

As will be shown in a later section, the distal tips of the longitudinal veins are originally present in veinlet in stage P 2b, but very soon disappear. This circumstance provides an explanation for an interesting phenomenon, which, like the malformed dumpy wings mentioned above, seems to show that the onset of the tension in dumpy wings may be shifted in relation to the time of appearance of the veins. In some $dp^{02}ve$ wings the tip of L 3, as far as it remains, is bent downwards as it would be in a normal dumpy; one can make out on the margin of the wing the place where the vein should meet it, and one can see that the missing part of the vein forms a smooth curve, just as though it had originally been present, and was distorted by the tension (Text-fig. 6f). In other specimens, however, the vein L 3 ends abruptly, with no downward curvature (Text-fig. 6e). This suggests two conclusions: first, that the tip of the vein had already disappeared before the tension had developed sufficiently to cause much distortion; and secondly, as a corollary, that the tension is more effective when acting on a closed system of veins, such as is provided by L 3 and the margin when they are united, than when acting on an open system, such as that of L 3 ending blindly in the epithelium. This latter deduction echoes the earlier suggestion that it is difficult to believe that the distortion of the veins is produced by stresses transmitted entirely through the epithelia.

The fact that a contraction of the epithelia is involved in the develop-

ment of dumpy is obvious from mere inspection. Now Dobzhansky (1929) showed some time ago that in the adult wing each membrane is only one cell thick, and each cell carries a single hair. By counting the number of hairs in a given area, it is therefore possible to determine the average area of the cells. As would be expected, the area is smaller in dumpy wings than in normal ones. In fairly extreme dumpies the number of hairs per unit area in the region of the posterior cross-vein is about 1·8–1·9 times as great as it is in normal wings. It varies according to the grade of dumpy and according to the region of the wing. In the wings which were measured to get the figures just quoted, the reduction in the area of the cells just accounted for the reduction in the total area of the wing, as compared with normal. Although, as has been said, the reduction in cell size is not uniform, and the ratio given above would therefore not hold accurately over the whole surface, this variation would only give rise to a second-order correction, and the general agreement in cell size and wing size can be taken as strong evidence that in the development of dumpy there is no actual loss of cells; the effect is a deformation and not a degeneration and disappearance of part of the structure.

The method of counting the hairs is particularly interesting in connexion with the question of whether, during the distortion of the wing, the veins are actually shifted relative to the epithelium, or whether, on the other hand, the veins and epithelium are merely distorted as a coherent structure. The places where one might look for a possible effect of this kind are chiefly at the distal end of the space between L 2 and L 3, and in the region of the cross-veins, since it is in these two regions that the distortion is most noticeable. The latter region is the more favourable to exact study. It is a noticeable feature of dumpy that the distance between the intercepts of the cross-veins on L 4 is considerably shortened, not only absolutely, but also relatively. Thus if one measures along L 4 from the junction of L 4 and L 5 to the anterior cross-vein, from there to the posterior cross-vein, and from the posterior cross-vein to the margin, and expresses the lengths as percentages of the total length (Text-fig. 7), the figures for normal wings are about 30 : 20 : 50, whereas for fairly strong dumpies the figures are about 30 : 15 : 55. This shortening of the inter-cross-vein distance does not, however, fully describe the changes in shape, since there is also a considerable spreading out of the veins. Thus if one draws a line along the main direction of L 2 and one along the proximal part of L 5, the angle (θ in Text-fig. 7) between them is about 29° in wild-type wings, and about 36° in moderately
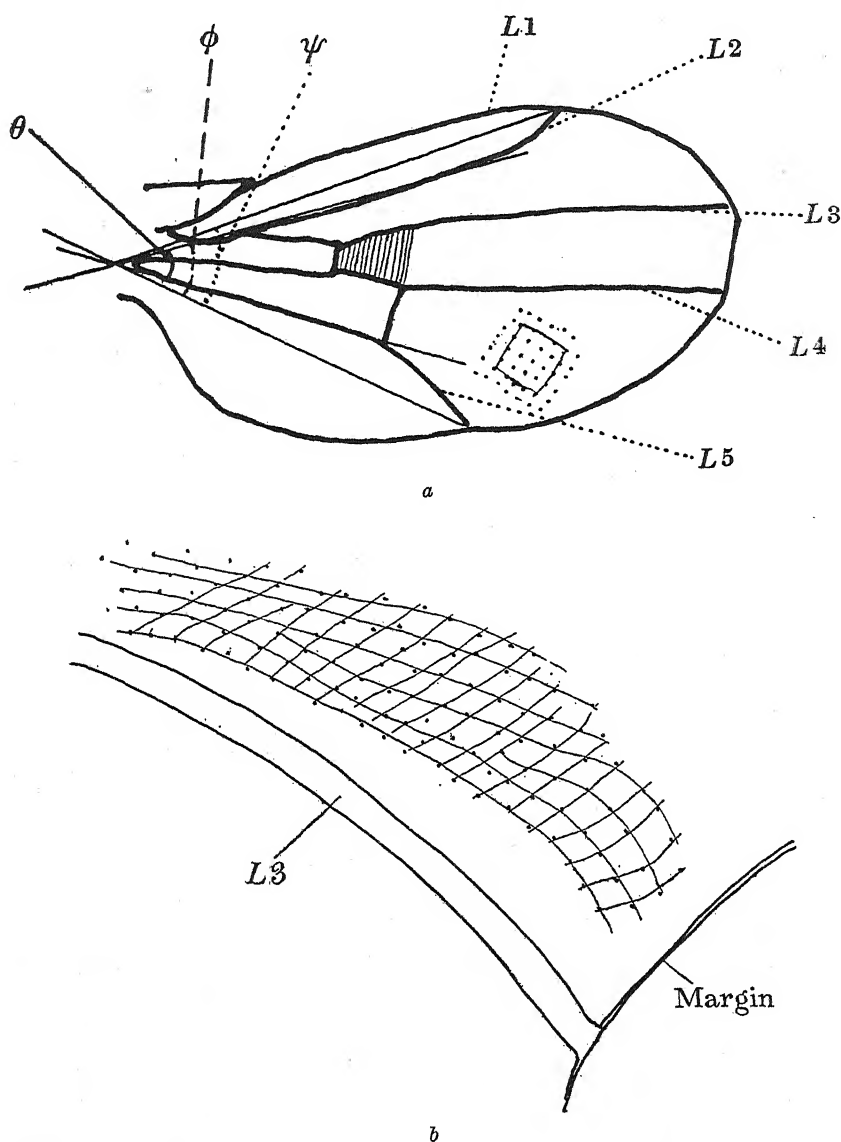
Text-fig. 7. *a*, wing of dachs, to show various dimensions measured. θ = angle between main directions of L 2 and L 5, ϕ = angle between L 2 and tip of L 5, ψ = angle between tips of L 2 and L 5. The shaded area is the "area bounded by L 4, the anterior cross-vein, L 5 and a prolongation of the posterior cross-vein". At the bottom right some hairs are drawn (at a magnified scale) to show the roughly linear arrangement, which forms parallelograms whose length and breadth were investigated in narrow and broad. *b*, hair distribution in dumpy, just anterior to L 3, showing the bending of the lines of hairs as they approach the margin.

dumpy wings. A greater difference is found if one measures the angle between the direction of L 2 and a line joining the intersection of the previous two lines to the marginal end of L 5 (ϕ in Text-fig. 7); in this case the angle for wild type is about 38° and for dumpy about 47°. The spreading effect is in fact more marked distally than it is proximally.

A similar spreading of the veins can be found in other mutants which show a shortened inter-cross-vein distance, such as dachs, approximated, etc., and this will be discussed later. In the case of dumpy we are dealing, as we have seen, with the deformation of a moderately elastic system of linear members, the veins. It is clear that the shortening of the inter-cross-vein distance and the spreading of the veins are causally connected as two aspects of the general deformation. The question arises as to whether such a deformation of a coherent structure is all that has occurred or whether the posterior cross-vein has actually shifted its position along L 5 and L 4, so as to approach the anterior cross-vein. If the vein system has merely been deformed as a coherent material structure the number of cells included in various regions between the veins should be the same in dumpy as it is in wild type, whereas if the veins slide along one another the number of cells should differ.

This is not a very easy point to determine. If one takes a large area of the wing, such as that included between the anterior cross-vein, L 4, L 5 and the margin, the area of the cells is not constant throughout it in such a type as dumpy, in which the deformation has a differential effect. On the other hand, there are no smaller areas which are at the same time considerably deformed and also strictly bounded. As a compromise one may consider an area bounded as follows: by the anterior cross-vein, L 3, L 4 and a line prolonging the posterior cross-vein to cut L 3 (cf. Text-fig. 7). The relative areas of these regions in a wild type and a dumpy wing which were measured were 1452 : 650 (i.e. nearly 22 : 10). In the same regions the relative areas of the cells were 81 : 45. The relative number of hairs in the two regions was therefore 1452/81 : 660/45, that is 100 : 82. In another case the figures came to 100 : 69. This denotes a reduction in number of cells of about 25 % in the dumpy. One can visualize what is involved if one imagines that, taking the region as it is in the wild type, the cells are reduced in area to the dumpy size, and the region is then deformed to the shape it has in dumpy, and that finally the posterior cross-vein is slid in towards the base of the wing sufficiently to reduce the number of cells in the region in question by 25 %. Now since the prolongation of the posterior cross-vein is longer than the anterior cross-vein, a reduction in cell number of 25 % will be achieved

if the posterior cross-vein moves somewhat less than 25 % of the distance between it and the anterior cross-vein. In fact, the amount of slide which is indicated is at most 20 % of the final dumpy inter-cross-vein distance. Another way of putting this is to say that the calculation indicates that before any sliding takes place, the normal inter-cross-vein distance would be shortened by 40 % of its length, owing to the deformation, and that sliding of the posterior cross-vein appears to be responsible for shortening it a further 10 %. Whether this result is really significant is somewhat doubtful; the limits of the areas to be compared are bound to be somewhat vague, since they are bounded by definite veins on only three sides. If, however, one accepts them at their face value, one should note that the sliding covers a length which is not much more than four times the width of the cross-vein in the adult wing, and would certainly be included within the width of the cross-vein when this is first formed in stage P 2. One may conclude that if any shifting of the cross-vein occurs, it goes on within the broad lacuna from which the adult vein develops, and is very definitely subsidiary to the main effect, which is a deformation. We shall see later (p. 119), however, that shifting of the cross-vein can occur at least in the early part of stage P 2: but perhaps this is before the dumpy tension is fully developed.

Owing to the absence of areas which can be delimited even as crudely as the one we have just considered, it is not possible to discuss the deformation of the submarginal cell (between L 2 and L 3) in a similar way. There is, however, another question which can be investigated in this region, which is not without interest, particularly in connexion with considerations which will be discussed later in relation to mutants such as broad, lanceolate, narrow, etc. The distribution of hairs can give us information not only as to the area of the cells but also as to their arrangement, and one may enquire how far this is affected by the distortion of the epithelium. Do the cells retain their connexions with one another, so that the deformation is entirely by stretching and compression of them, or do they slide over one another into a new arrangement?

A glance at the distribution of the hairs answers the question, at least partly in favour of the latter possibility. The hairs on the wing are arranged in rough rows. If no sliding took place, rows of hairs which normally continue out beyond the end of L 2 towards the tip of the normal wing would in dumpy have to be curved round, since, in that form, the wing tip does not extend beyond the end of L 2. It is clear that no such exaggerated curvature is found. On the other hand, the

distribution of the hairs still gives some evidence of strain, as can easily be seen if the hairs are joined up as symmetrically as possible in groups of four to make little diamonds; these are more extended from anterior to posterior in the more distal regions, where the deformation of the sub-marginal cell as a whole is greatest, and this is evidence that the cells themselves have been deformed here. We find, then, that the deformation of the epithelium takes place partly by sliding of the cells over one another and partly by stretching of the cells. One cannot determine, from the data available, what is the relative importance of these two processes in producing the force required to deform the veins.

The dumpy-Blade phenomenon in other mutants

The distortions of dumpy and Blade may, as we have seen, be attributed to abnormal relations between the contracting wing epithelia and the resistance of the veins. In both of these cases the contracting force of the epithelia is abnormally strong. It is interesting to enquire whether there is any sign of a similar interplay of forces in normal development, or whether the epithelia and veins are there so perfectly adjusted that they contract to exactly the same extent. It has been pointed out, in the description of normal development, that there are some changes in shape of the wing blade during the relevant stages (P 2), and these can presumably be at least partly attributed to forces of the kind which become abnormal in dumpy and Blade. The morphological changes involved are subtle and not very easily defined; there is some pointing of the distal tip of the wing, reminiscent of that seen in Blade, and probably correlated with the increasing strength of the anterior marginal vein; and there is a broadening of the proximal region posterior to L 5, correlated with the pinching together of the region which will form the wing articulation.

One is more impressed with the reality of these changes if one compares a normal wing with that of Delta-6, for instance. Regarding for the moment only the general shape, and paying no attention to the thickening of the veins, there is no doubt that the Delta wing has a peculiarly lax, slack appearance, which is exactly what one would expect if the epithelial tension had been abnormally low.

Very definite evidence that a dumpy-Blade process is involved in the development of all types of wings is provided by some of the scalloped wing types. It is clear that if large pieces of the wing are removed, while the pattern of veins remains unaltered, the balance between epithelium and vein will be altered. This is exactly what happens in some of the

scalloped types, and the occurrence of a distortion in these types shows that the epithelial tension and vein resistance are in fact present there just as they are in dumpy and Blade.

Since in these types the wing margin is not entire, the distortion must be judged from measurements which relate only to the veins and have no reference to the margin. The best index of this kind is the angle between the main directions of L 2 and L 5 (θ). In the wings in which pieces are removed from the anterior and posterior margins, it is found that as the wing becomes more narrow and elongated, this angle becomes smaller. Thus in a normal wing it is about 29–30°; in Lyra, in which both anterior and posterior margins are removed, it is reduced to about 25–26°; in Bx^c it may be reduced further, to 22° (Text-fig. 6 j, k, l). Study of the figures of some of the other narrow scalloped wings will show that in very many of them there is a tendency for the angle θ to be smaller, that is to say, for the narrowness of the wing to be exaggerated. While I am not prepared to say that the reduction in the angle is quantitatively proportional to the narrowing which has been previously produced by the scalloping, it is clear that there is a real correlation between the shape of the wing before contraction and the kind of distortion which is produced. This comes out very well in wings such as those of vestigial-nipped, in which the position of the scalloping is somewhat variable (Text-fig. 6 h, i). In some wings which have lost only the distal end the angle may be normal or even slightly larger than normal, while in others with longitudinal scalloping the usual reduction in the angle occurs. In Xasta a deep notch is removed from the tip of the wing, which is thus somewhat squarer than normal; and correlated with this the angle θ is increased to about 33–36°. In vestigial only the base of the wing is represented, and, owing to the peculiar conditions of contraction of this region, its shape cannot be directly compared with that of a more nearly complete wing. It behaves, however, as one would expect a broad rounded wing to behave; that is to say, the veins are somewhat splayed out instead of being squeezed together. The best indication of this is the wide angle between L 3 and L 4, the anterior cross-vein being, when present, considerably longer than normal and running along the margin (Text-fig. 6g).

The Blade-like elongation of already narrow wings, and the corresponding dumpy-like broadening of rounded wings, seem reasonable enough mechanically. Clearly we do not know enough to draw any detailed deductions from the phenomena, but in general terms they seem to indicate that the epithelial tension increases with increasing curvature, and is strongest along the line of maximum curvature. Thus in the narrow

wings, the radius of curvature in the transverse plane must be relatively shorter, in the inflated stage, than it is in wider wings. This consideration may also explain why Blade wings can develop in such very different ways even in the same fly; if the wing once starts to elongate, it must thereby become narrower, and its radius of curvature in the transverse plane shorter, so that the tendency for narrowing and elongation will be increased, whereas of course if it starts by becoming broader and shorter, an exactly opposite set of phenomena will occur which will tend to reinforce the dumpy effect.

Again, the suggestion that the epithelial force may be related to the curvature of the surface suggests the possibility that abnormal tensions may be produced in particular regions of the wing in which the shape has been altered. The conditions for such abnormal local forces are given in certain of the scalloped types, where pieces of the wing may be apparently bitten away. We do indeed find in these wings local differences in the contraction; that is to say, a wing which is more or less cylindrical in the inflated stage may contract down to a blade with quite an irregular margin. The changes of shape which would be produced during the inflation and contraction of the wing would, however, depend in a complicated way on the elastic properties of the epithelium, and the radii of curvature in different planes, and no useful purpose would be served by discussing various possibilities at this stage.

Summary

In dumpy and similar mutants the entire wing is distorted as compared with normal, as can be seen from the venation, particularly by the widening of the angle between L 2 and L 5 and by the shortening of the inter-cross-vein distance. During development the distortion appears during the contraction from the inflated stage. It involves an abnormally great contraction, since the area of the wing is reduced in comparison with normal. The area of the cells is correspondingly reduced, so that no cells are lost by degeneration.

The contraction is not dependent on the margin, since it is operative in scalloped types from which the margin is absent. Nor is it dependent on the veins, since these may be too long for the epithelia instead of vice versa; moreover, the contraction occurs normally in dumpy-veinlet wings in which the connexion between the veins and the margin is broken. On the other hand, all the facts are consistent with the hypothesis that the contraction is primarily due to the epithelia.

The distribution of hairs shows that the contraction occurs partly by the sliding of cells relative to one another and partly by the elastic deformation of the cells.

The developing veins act as stiffening rods within the contracting epithelia. They can be altered in length, and the whole system can be splayed out or squeezed together; they are only slightly, if at all, shifted in relation to the cells in the region of the posterior cross-vein. The stage of maximum contraction can occur at different times in relation to the stiffening of the veins; thus sometimes the veins are already too stiff to be adequately deformed, and remain too long for the epithelium. Similarly in dumpy-veinlet wings the distal end of L 2 is sometimes affected by the contraction and sometimes not.

An increased contraction of the epithelium can also lead to an elongation of the wing, as in Blade (of *D. pseudo-obscura*). The choice between a Blade type or a dumpy type of contraction may perhaps be related to the degree of elasticity of L 1 at the crucial stage; in any case the choice rests on slight variations in conditions, since both types of wing may occur on the same individual.

Elongation by a contractive mechanism occurs in all wings which are abnormally long in shape at the time of the contraction, for instance, in some of the scalloped types such as Lyra, Beadex, etc. Abnormally short types, such as Xasta and vestigial, undergo dumpy-like shortening.

It is probable that the tension developed in the contracting epithelium is related to the radius of curvature of the partly inflated wing sac.

Scalloping genes

A very considerable number of genes are known which produce wings which look, in a rough way, much like normal wings from which pieces have been mechanically removed, as it might be with a pair of scissors. One need only mention the alleles of vestigial, cut. Beadex, Lyra, scalloped, etc. The development of mutants of this type has been studied by Chen (1929), Auerbach (1936), and particularly by Goldschmidt (1935, 1937). None of these authors, however, followed the pupal development right through in a continuous series, and it is doubtful whether the interpretations which have been put on the appearances which have been noticed can be allowed to stand.

The author who has discussed the development of these mutants most thoroughly is Goldschmidt. His fundamental point is that, if one examines the development of the slighter grades of notching, one finds that the wing is normal and complete at an early stage, and that the

notches are produced by a later degeneration, which causes the disappearance of certain material from the margin. If a series of different grades are studied it will be found that not only does the degeneration extend farther in from the margin, but also that it starts earlier, so that in extreme scalloping the effect is supposed to be visible at the time of pupation. The latter point had already been stated by Chen for vestigial and was confirmed by Auerbach for vestigial and Pointedoid. In these extreme forms, however, little has been made out as to the mechanism of the effect; the evidence that we are dealing with a degeneration comes from a study of the less extreme forms, and this evidence must now be examined.

When one compares the photographs published by Goldschmidt with the developmental stages described and figured above it is clear that the earliest stage which he figures is that of the inflated bag (PP 4 and P 1). The series of wings in which the notches can be seen to appear belong to the successive phases of stage P 2. Now it has been shown that during this stage the normal wing undergoes a contraction by which the hollow inflated sac of stage P 1 becomes a flat wing-blade. As soon as this is realized, a new interpretation for the gradual appearance of the notches becomes possible and requires investigation. One must consider whether the progressive incision of the notch is not due merely to the deflation of an already notched sac. It is a well-known phenomenon that if one inflates an irregularly shaped elastic bag (such, for instance, as a toy rubber balloon in the form of a doll), the irregularities of the surface disappear as the bag becomes more inflated, and appear again as it collapses.

An examination of certain favourable cases makes it clear that this phenomenon is at any rate concerned in the wing development. For instance, the stock Xasta has a wing from which a large distal area is missing (Pl. 4, fig. 4). In bag stages this lack can be discerned, by the flatness of the distal end of the sac (Pl. 4, fig. 3), but it is by no means so marked as it becomes in the adult, and a series of stages can be found paralleling the phenomena described by Goldschmidt in other mutants. When, however, one examines the prepupal wings, from stages earlier than the bag stage, one finds a very marked distal notch (Pl. 4, fig. 2). Clearly the notching becomes less obvious in the inflated stage, and the appearance of the wing in that stage is not a safe guide to the actual state of affairs.

Xasta causes a very marked change in the shape of the general outline of the wing, and is therefore particularly easy to detect even in the

tiny prepupal wings. The lower grades of distal notching could not be expected to be so obvious, and it is extremely likely that they could be detected, if at all, only by refined statistical analysis of exactly comparable stages. Similarly, the more usual marginal scallopings which cause comparatively slight changes in general outline will not be very easy to detect. Probably the slightest which has been as yet detected in the prepupal wing is cut-6 (Pl. 4, figs. 9–13). This causes the loss of parts of the anterior and posterior margins, and a certain pointing of the wing tip. Goldschmidt considered it one of the strongest scallopings which remained normal up to his "early pupal stage", that is to say up to our bag stage. Actually, however, it is narrower than normal in the bag stage, although the distal end is well rounded. Moreover, it is some-
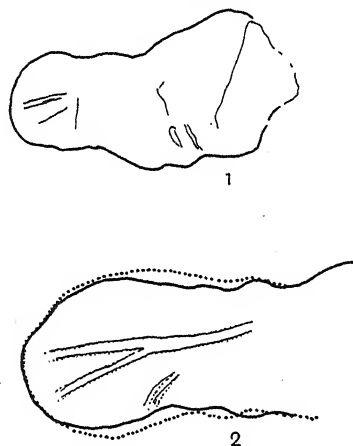
Text-fig. 8. Lyra. 1, outline of stage P 2; 2, stage P 3 (full lines) superposed on normal (dotted lines) of same stage.

what narrower than comparable normal wings even in prepupal stages. Here again then the observed appearance of the scalloping in the contraction stages is at least to some extent deceptive, since a certain amount of this scalloping must be present earlier. A similar prepupal scalloping can also be seen in other mutants of the same type, such as Lyra (Text-fig. 8), and is, of course, more obvious in the still more extreme forms such as Bx^J, etc., in which it has also been noticed by Goldschmidt.

With the demonstration that the deflation accounts for some of the apparent degeneration, it becomes natural to ask whether it does not in fact account for it all. Is there in fact any actual degeneration subsequent to the inflated stage? No histological demonstration of it was offered by Goldschmidt, although he drew attention to the appearance

of the edge of the wing in the scalloped region in whole mounts. However, one must remember that the wing is a fairly thick object at this time, and that in looking at the edge of a whole mount one is taking a tangential view of a fold. The breadth of the dark band which runs along the edge will therefore be very dependent on the exact conditions of the fold and degree of inflation. If, as has been suggested above, the wing actually has an irregular shape, and if these irregularities are now appearing after being obliterated by the inflation, we should expect that the conditions would be different at different points along the edge, and that the marginal dark band would vary in width. Even at the highest magnifications I have not seen any positive evidence of degeneration in whole mounts, and an examination of sections leads to the same conclusion. It might be, however, that the histological picture of degeneration was not easy to recognize, and we must therefore devote further consideration to the indirect evidence of degeneration, which provided the basis for Goldschmidt's hypothesis. This evidence is perhaps most striking in the case of vestigial itself. From Goldschmidt's figures, two main points emerge. In early stages one can see a slight knob on the tip of the wing which seems to be on the point of being broken away and lost; and again one notes that in late stages the wing rudiment occupies a smaller proportion of the enveloping chitinous sac than normal. Both these appearances suggest a degeneration (Pl. 4, figs. 15–17).

The terminal knob is an extremely regular feature of vestigial wings. It is most marked in the inflation stages, and at that time it is often connected only by a narrow bridge with the rest of the wing. The chitinous sheath here cuts deep into the outline, and it certainly looks plausible to suggest that the whole knob may be as it were strangulated during the contraction, and torn loose. However, an extensive search has failed to reveal any traces of such cast-off knobs; and it seems certain that if they are in fact cast off they should be found, since there is no circulation within the wing sheath which might remove the remnants of them. Again, it is noticeable that in later stages, when one would have to imagine that the casting-off had already occurred, the wing still shows a slight terminal bulge, although this is much less sharply constricted off than the earlier knob. This suggests rather that the knob is later withdrawn more or less into the main body of the wing.

My own preparations again confirm the second point of Goldschmidt's figures, namely, that the late vestigial wing occupies an unusually small proportion of the pupal sheath. Before one can evaluate this fact, there are two main points to be borne in mind. The first is that the degree of

contraction of the wing is not the same in all regions. As was shown earlier, the base of the wing contracts much more than the tip. Now the vestigial wing comprises only the most basal part of the normal wing, and is made up very largely of vein tissue which in the adult is much smaller celled than is the membrane. It would therefore be expected to contract much more than would a complete wing, and therefore to bear a different relation to the pupal sheath.

The second point is also concerned with the degree of contraction of the wing. The veins of the vestigial wing can easily be identified with those of the normal wing, and if the patterns are compared it is clear that the vestigial is deformed, in much the same way as dumpy is deformed. Thus the central vein of vestigial must be L 3, and in fact it bears the characteristic chorotonal organs. Moreover, this vein is often prolonged posteriorly round the margin (where it should perhaps be regarded as representing the anterior cross-vein. It is clear that the whole vein system has been to some extent spread out, and with the example of dumpy in mind one naturally thinks of an abnormal contraction as the cause of this. The suggestion is confirmed if one examines the size of the cells of the intervein membrane in vestigial. It is considerably less than in wild type, typical figures being 4·6, 4·54 and 4·3 hairs per unit area, as compared with a figure for wild-type wings of about 2·8. Thus the area of the vestigial cells is only about 60 % that of normal cells.

We have therefore two well-attested and definite reasons why the late vestigial wing should occupy an abnormally small space inside the pupal sheath. Again the question arises whether it is necessary to postulate any degeneration of the margin in addition. Unfortunately it is extremely difficult to give any quantitative estimate of the extent of the contraction of the proximal region of the wing in a longitudinal direction. In the transverse direction, however, the contraction from the fully inflated stage to the definite wing stage in which the wing articulation is complete is about from 100 to 30 for the most basal regions. The additional contraction by a "dumpy" phenomenon, which we have seen to occur in vestigial, would bring this down to a contraction from 100 to about 18. The maximum transverse contraction even of the main blade of a vestigial wing is of the order of 100 to 40. There seems no reason, therefore, to postulate any additional minifying effect such as a marginal degeneration.

The foregoing discussion can be applied, *mutatis mutandis*, to the development of other mutants of the scalloped type. It is only in the

extreme types of scalloping, in which only the basal region of wing is retained, that the excessive contraction of this region during pupal development plays a part in simulating degeneration. In the less extreme forms this appearance is brought about partly by the tendency for re-entrant angles to be smoothed out in the inflated stage, as we saw in Xasta, and partly by the occurrence of the dumpy phenomenon. The latter is particularly marked in wings which deviate largely from the crude overall shape of a normal wing. In the elongated types such as Beadex, for instance, the elongation is exaggerated in the adult, as is shown by the acute angle between the longitudinal veins. In broader types, which more nearly approach the normal shape, the effect is less noticeable (see previous section).

It appears then that there is no necessity to attempt to explain the phenomena of pupal development of scalloped wings by postulating a marginal degeneration. Before this hypothesis can be rejected, however, it is desirable to replace it by some other which can equally well account for the remarkable shape of the adult wings. The general impression given by a scalloped wing—that it is a more or less normal wing from which pieces are missing—is really very surprising. One is bound to conclude that the scalloping takes place after the main determination of the veins, since even if deformations of the vein system, or possibly minor shifts such as that of the posterior cross-vein, may be considered possible, it is clear that major readjustments of the longitudinal veins to the new wing shape do not occur. Now in the extreme types of scalloping the first effect on wing shape is noticeable very early, even before the pupal period. Goldschmidt and Auerbach have both pointed out that in vestigial, for instance, the region of the imaginal bud which becomes invaginated to form the wing fold is smaller than normal from its very first appearance (Pl. 4, fig. 18; Text-fig. 9). This can also be shown to be true for several other mutants. Thus in Lyra and cut-6, the invagination is from the very first narrower than in wild type, and in Xasta the tip of the invaginating fold is truncated (Pl. 4, fig. 1; Text-fig. 9). At this early stage, the abnormalities in the shapes of the mutant wings are very much less obvious than they later become. But, in describing the normal development, it was shown that as the wing fold is everted, it is also protruded and stretched (p. 78), and it is clear that, if one tries to project the later wing back on to the early wing fold, one would have to imagine it squeezed up longitudinally; one is reminded of the map of presumptive areas of the amphibian gastrula, in which the presumptive notochord, for instance, occupies a much broader and shorter area than it does later
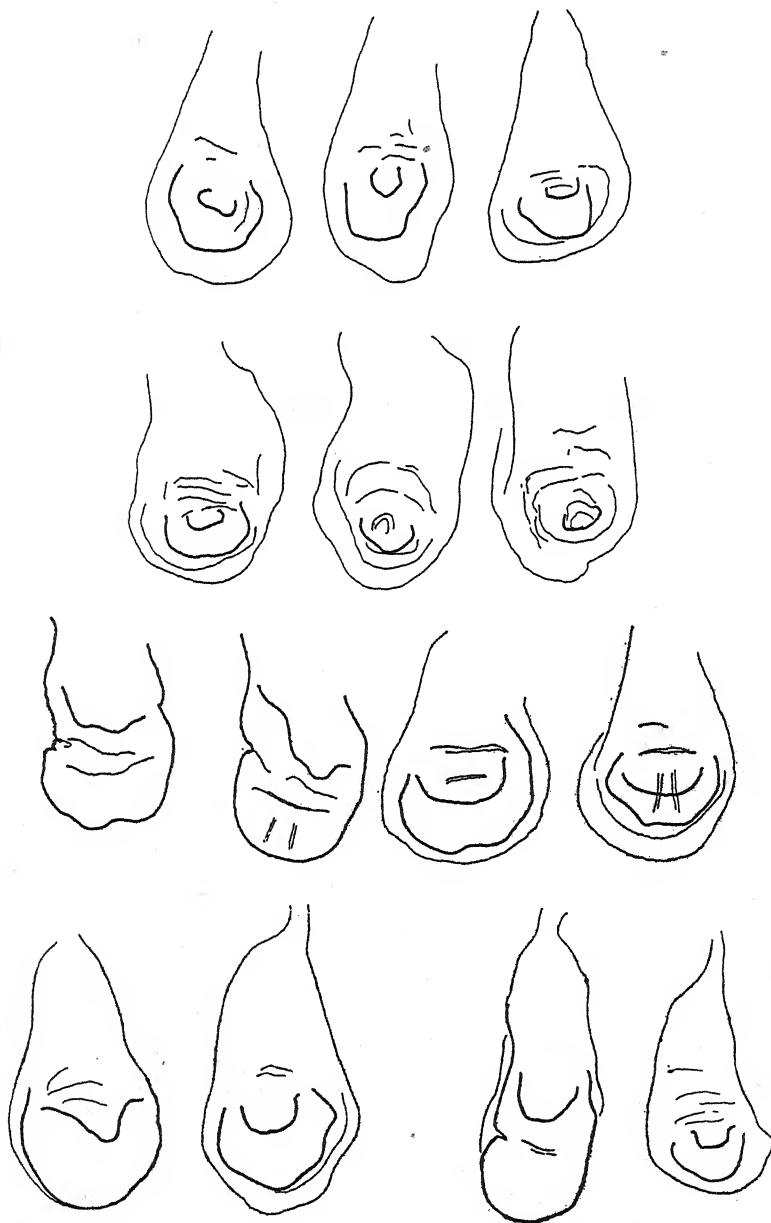
Text-fig. 9. Imaginal buds. Upper row, Beadex-J; second row, vestigial; third row, Xasta; fourth row, two normals on left and two vestigial-nipped on right. Note narrow wing-fold in Beadex-J and vestigial-nipped, small fold in vestigial, fold of normal breadth with indented tip in Xasta.

on. It is only natural that the peculiarities of the mutant shapes are less obvious in this highly condensed stage.

Since the wing shape is already altered at this time, and since the characteristic feature of these mutants is that the venation is not adjusted to the altered shapes, it is clear that the course of the veins is already determined at the time when the invagination takes place. That is to say, the veins are already determined at the time when the folding brings their upper and lower surfaces together. But we have just seen that the folding is not always in the same position; in Beadex the fold is narrow, in Xasta broad and so on. If one supposes that both the upper and the lower surfaces of the veins are equally determined, it is inevitable that with these variations in folding the two surfaces would not always be brought into perfect apposition, so that one would expect to find parts of veins on the upper surface dissociated from those on the lower. This never occurs, either in the scalloped types or in any others, such as dumpy, in which the veins develop under considerable tension (bloated is a possible exception, but here there is no doubt that the veins have been secondarily disrupted, see p. 129). One must conclude that only one surface of the venation is determined, either the upper or the lower, and that this then impresses its own pattern on the other surface by some sort of inductive mechanism.

Moreover, if it is, for instance, the upper surface which is determined, and if, by some change in the shape of the folding, tissue which is normally part of the upper surface is brought on to the lower surface, it is clear that it behaves like lower-surface material, that is to say, its own veins do not develop but it can form veins if called upon to do so by the upper surface.

It is not easy to see any other alternative to these conclusions, but the idea of a determination of one surface, an inductive relation between the two surfaces, and the inability of the determined veins to continue their development if they arrive on the wrong surface, may appear somewhat fantastic and unlikely. Fortunately, one can point to a visible feature of the wing bud which would apparently exactly fulfil the requirements. It was pointed out in discussing normal development (p. 80) that in the earliest stage of their appearance the veins are represented by wider spaces corresponding to weak ridges on the upper surface of the wing fold. If one supposes that these ridges are, by the very fact of being ridges, thereby determined as vein surface, everything falls into place; they are originally on one surface only (the upper); they do have a differential relation to the lower surface, by failing to come in contact

with it as early as does material between the ridges; and finally, if the fold appeared in the wrong relation to them, they would clearly be obliterated before they reached the lower surface, since they would have to pass round the sharp curvature at the apex of the fold. It will not be easy to apply a direct test to discover whether the longitudinal ridging of the wing fold is in fact the process by which the veins are determined, but one may say that this hypothesis satisfactorily accounts for all the data derived from the study of mutant wings (and also that from such experimental operations as have yet been performed, which will be made the subject of a further communication at a later date).

We have here reached the fullest explanation which is, I think, as yet possible of the wing scalloping. The appearance of the adult wings shows that the venation is determined before the shape of the wing is altered, that is to say, before the folding takes place; and the folding can be observed to be different in different mutants; it inevitably follows that the fold must cut out different regions of the determined pattern of veins.

We can therefore express the action of all the scalloping genes by saying that they change the relation between the processes which determine the position and shape of the invagination and those which determine the venation system. This provides a single unified explanation of the action of genes of all degrees of severity. It also fits in well with the scanty experimental evidence. This mainly relates to the somewhat obscure phenomenon of the sensitive period. Stanley (1935) and Harnly (1936) have shown that the sensitive period during which temperature shocks will affect the expression of homozygous vestigial (in the direction of larger wings) is in the early part of the third larval instar. This is about the time the invagination starts. Again the sensitive period for the production of phenocopic scalloping of wild-type wings is late in the third instar (see Goldschmidt, 1939), that is to say, at the time when the invagination is beginning.

It would be very interesting to know the mechanism by which the genes alter the relative position of the wing fold, but, as in nearly all investigations on the developmental effects of genes, it is impossible to trace the causal sequence right back to the gene itself in an unbroken succession. At present there are no data as to what occurs in the imaginal bud before the folding, although it is clear that the thickened area which becomes invaginated is reduced in size in vestigial buds and probably in many others of the scalloped types. It may be that an alteration in size has little effect on the longitudinal grooving, but shifts the position of the

wing fold in relation to the whole thickened area. If this were so, it might in itself be enough to account for the whole phenomenon, but the data as to this are not yet to hand; the matter is under investigation.

Goldschmidt has, in general terms, applied his hypothesis of degeneration to the events during larval life.

This extension of the hypothesis does not, however, stand on its own feet, since there is no independent evidence that degeneration occurs in the larval stages; its only value is to provide a uniform explanation of all grades of scalloping, and it becomes quite unnecessary if we reject the evidence for a degeneration in the pupal period. It is, moreover, extremely difficult to see how a degeneration could possibly account for the facts. If, for instance, the tip end of Xasta is supposed to have partially degenerated before the folding occurs, one would have to imagine that, in a coherent sheet of tissue, a certain region bodily disappeared. Similarly, any other wing margin, such as those attacked in cut-6, would before the folding consist of a flat area within a larger sheet. Since the regions of the wing which do not disappear are not deformed, it would seem that the degeneration would have to leave holes; a most unplausible idea.

Summary

The data of this section may be summarized as follows:

There is no histological evidence of the degeneration of the wing margin which has been supposed to occur during the pupal period. At the time at which the apparent degeneration can be seen, the wing is contracting from an inflated condition such as is also found in the development of wild type. In various types of scalloped wings the reduction in area may be exaggerated by one or more of three secondary effects: (1) re-entrant angles tend to be obliterated in the inflated stage and to appear again on deflation; (2) abnormal contractions of the dumpy type occur in wings with an abnormal shape; (3) in some of the extreme scalloped types only the base of the wing is left, and this normally contracts more than does the rest of the wing. It is shown that these effects are probably quantitatively sufficient to produce the observed reduction in area.

The first effect of the scalloping genes which has been seen is an alteration in the size and shape of the fold along which the wing is invaginated into the imaginal bud. This leads directly to a wing of an abnormal shape; and to this new shape the veins do not adjust themselves.

Two consequences are deduced, namely, that in the late larval imaginal bud the veins of one wing surface or the other, but not of both, are already determined; and that if a determined vein surface arrives, in consequence of abnormal folding, on to the other surface of the wing, it does not develop as a vein surface.

These two characteristics of the determined vein system actually characterize the longitudinal ridges on the upper surface of the evaginating wing fold, which are the earliest signs of the veins which have yet been made out. All the phenomena of scalloping can therefore be accounted for by the hypothesis that the ridging of the upper surface of the wing fold determines that the ridges shall develop as vein surface, and that the scalloping genes have little or no effect on this process, but alter the position of the fold relative to that of the ridges.

The scalloping genes whose development was studied were: vg, vg^{np}, Bx^J, Bx^c, Bx^3, Ly, Xa, ct-6, sd.

THE VEIN SYSTEM

The veins of a *Drosophila* wing are an extremely simple example of an animal pattern. They form a more or less two-dimensional network of lines, enclosed within an area which is bounded by the margin of the wing. As we shall find, all the veins, excluding the marginal vein L 1, may behave as a unit, which suffers a general distortion in some mutant types, while the margin as a whole, including L 1, behaves as another unit, which in these cases is very little affected. In an earlier section devoted to the dumpy-Blade phenomenon, we had examples of general distortions of the whole vein system including the margins, but in those cases the distortion was a mechanical one exerted by the contracting epithelium on an already determined and partially differentiated set of veins. The distortions which will now be considered occur at a much earlier period, in some cases during the process of determination of the veins. The fact that general distortions of the whole pattern can be produced at such early stages shows that during their determination the veins are not independent units; they form a single pattern, or field, all parts of which are causally related to all other parts. This interconnectedness later vanishes, and we shall find that genes whose effects can only be detected in later stages of development have a more localized action. The conditions are, in fact, exactly those which we are used to in experimental embryology; an early regulative period of "field" action passes gradually into a stage of mosaic development in which each part is an autonomous unit.

The general nature of the distortions produced by some genes does not appear to have been explicitly recognized in the descriptions which have been published. An example is shifted-2 (Text-fig. 10). The most obvious effect is a narrowing of the space between L 3 and L 4, so that these veins are abnormally near one another throughout their length. If, however, a wing of shifted-2 is superposed on one of wild type, it will be seen that the effect is more general. All the veins, including L 2 and L 5, are pinched in so as to diverge at a smaller angle. The effect is a graded one, since it is the region between L 3 and L 4 which is actually
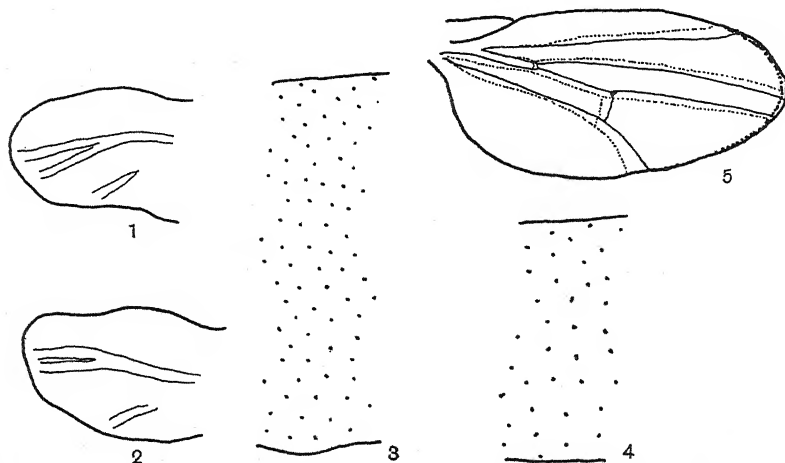
Text-fig. 10. Shifted-2. 1, normal wing of stage PP 3; 2, shifted-2 wing of same stage; note acute angle between the branches of the main vein; 3, hair distribution between L 3 and L 4 in a normal wing; 4, hair distribution between L 3 and L 4 in shifted-2; note that there are fewer hairs, farther apart; 5, adult wing of shifted-2 (full lines) superposed on normal (dotted).

affected most strongly. One can tell even from the adult wing that the effect is not a distortion of the already formed veins, since not only is the margin not distorted correspondingly but the number of cells between L 3 and L 4 is much smaller than normal, as is apparent from counting the hairs. In fact the number of cells is even smaller than would be at first suspected, since the cells in this region are larger than normal; it looks as though the pinching together had been at first very marked, and that at a fairly late stage there is some tendency to restore the normal pattern which has led to a stretching of these cells. Be this as it may, the main effect is a shift of the vein system relative to the epithelium, and this shift has applied to the system as a whole. When the development of the wings is studied, the abnormality can be detected very early,

in the young prepupal stage, in which the two branches of the central vein diverge at a very small angle. There is no evidence for any distortion of the wing at this stage either, and all the evidence points to the conclusion that the veins are distorted from their earliest appearance.

The series of genes dachs, four-jointed and approximated, have very similar effects not only on the legs (Waddington, 1940 *a*, *b*) but also on the venation. They cause a shortening of the distance between the two cross-veins. Again, this effect is only the most obvious one. In all these
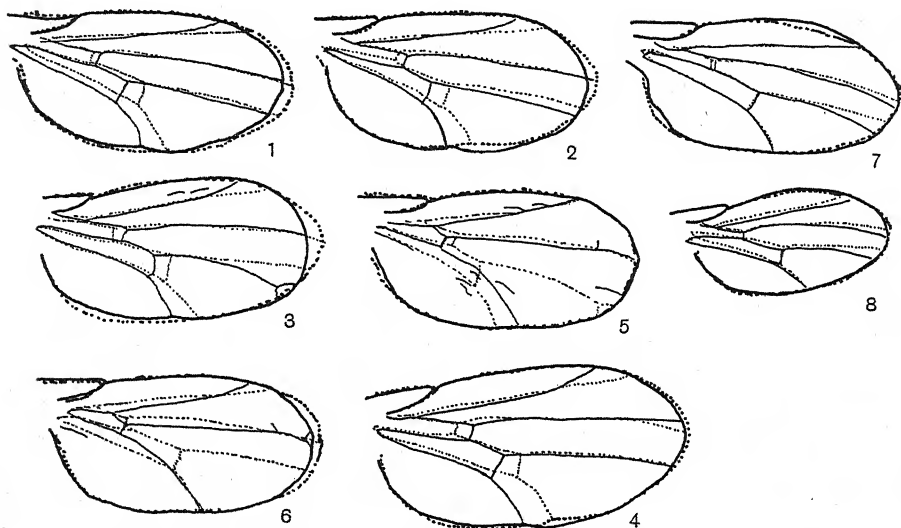
Text-fig. 11. Adult wings. 1, approximated (full lines) superposed on normal (dotted); 2, dachs on normal; 3, four-jointed plexus on normal; 4, approximated-dachs on normal; 5, four-jointed-plexus-cubitus interruptus on four-jointed plexus (note shifting of L 5); 6, four-jointed-plexus-cubitus interruptus on normal (the combination has brought L 5 back to its normal position); 7, dusky on normal; 8, miniature on normal (note disproportionate width of wing base). All the mutant wings drawn to same scale, and the normal wings adjusted in size to be equivalent to the mutant under consideration.

types the angle between L 2 and L 5 is slightly greater than normal, so that the veins are splayed out (Text-fig. 11). If one measures the angle (θ) between the main directions of these two veins, it is about 29–30° in wild type and 32–33° in dachs, approximated and four-jointed. The splaying, however, is stronger at the distal ends of the veins, and if the intercepts of L 2 and L 5 with the margin are connected to the intersection of their main directions, the angle between the two new lines (ψ in Text-fig. 7) is about 37° in wild and 43–45° in the others. The point is clear enough in the superposition of the mutant wings on normals

of the same size; and this is perhaps a better test, since the mutant wings are all smaller than normal, and systematic errors in comparisons of angles due to differential contraction of the distal and proximal regions, which will be described in miniature and dusky wings, will also enter here.

The superposition also makes it clear that the alteration in the position of the cross-veins is a real shift relative to the epithelium. The point can be checked in the same way as was done with dumpy. In these cases also, as has been stated, the wings are smaller than normal, but here, in contrast to dumpy, the reduction in size is attained without any great change in shape. The number of cells in the area bounded by L 3, the anterior cross-vein, L 4 and the prolongation of the posterior cross-vein works out at 37 % of the number in a wild type. The shift is therefore a real one, and the same is certainly true in the other two mutants under consideration.

It may be remarked that the compounds of approximated with dachs and four-jointed have been made, and that they possess wings which are indistinguishable from those of single genes. There is no reinforcement, either additive or proportional, such as Csik (1934) found for certain other wing genes. This behaviour is paralleled by that of the leg effects of these genes, and is to be explained in the same terms (see Waddington, 1940 a, b).

The development of these mutants has not been fully studied, but their effects would be extremely difficult to discern in early stages, since the cross-veins are lacking in the prepupal stages, and the slight divergence of the longitudinal veins could hardly be detected. However, as soon as the posterior cross-vein can be clearly made out in the pupal period, its position can be seen to be abnormal. One might therefore consider the possibility that these genes act during the very earliest stages of the contraction in P 2 somewhat before the dumpy process. The splaying of the veins and the blunting of the end of the wing might suggest that the margin, which is the first-contracted part in P 2a, expands distally and contracts proximally in such a way as to push the ends of L 2 and L 5 away from one another. But this suggestion is made rather unplausible by the fact that the characteristic splaying of the veins also occurs in the compound $fj\ ct^6$ in which the margin is lacking. It seems most probable, then, that the approximated, dachs, four-jointed pattern is determined before the beginning of the pupal period, and very likely before the eversion of the wing, that is to say, at the same time as the shifted pattern.

The problem of the time at which the effect on the cross-vein is produced will be discussed later (p. 132).

Summary

There are some genes which produce a general deformation of the veins without much effect on the margin; the possibility of such an action shows that the whole set of longitudinal veins, at least, forms a single field. One of the genes investigated, shifted-2, can be detected as soon as the veins are visible in the early prepupal stage, and must cause the original determination of the veins in the imaginal bud to occur in an abnormal pattern.

It is shown that approximated, four-jointed and dachs, which were known to cause a shortening of the inter-cross-vein distance, also cause a spreading out of the longitudinal veins, which is probably prior to the effect on the cross-veins. This does not seem to be a result of increased contraction of the epithelia, since it does not follow the dumpy pattern; nor is it an effect of differential contraction of the margin, since it occurs in cut-6 flies in which the margin is partly missing. It is most probably an effect exerted on the pattern of the veins at their determination, as in shifted-2.

The effect on the cross-veins is a real shifting of them in relation to the cells of the membrane, not a mere mechanical distortion of the normal pattern. The time at which this action is effective will be considered later (p. 132).

DEFORMATIONS OF THE WHOLE WING

Certain genes are known which cause deformations of the whole wing in such a way that the venation fits into the new margin. We have seen some examples of this in dumpy, Blade, etc., but the genes to be considered now produce much less abnormal effects. Moreover, in many cases the characteristic shapes which they produce can be detected earlier than the occurrence of the dumpy process, being apparent already in the prepupal stages.

The simplest genes of the type at present under consideration are miniature and dusky (*dy*) (Text-fig. 11), both of which cause the production of wings which are considerably smaller than normal. As Dobzhansky (1929) showed, the reduction in size in miniature is paralleled, and presumably caused by, a reduction in the size of the cells of the adult, as judged by the number of hairs in unit area; the same is true of dusky, in which the reduction in size is not so large, and is usually more

variable. Goldschmidt asserted that miniature wings are normal in size until the end of stage P 2, and that the small size of the adult wing is due to a lesser expansion during the following stages. In my material, however, the wings were slightly smaller even at the inflated stage, and remain so thereafter. The cells of the epithelia are also somewhat smaller at the end of stage P 2. The reduction in size does not, however, appear to be so great as that which can be seen in the adult wings. Thus the length of a fairly typical miniature just before stage P 3 was about 73 % of that of a normal wing of the same age, while an adult miniature was only about 52 % of the adult normal. It seems probable then that in miniature the cells are not only from the beginning smaller than in wild type, but that they also expand less, as Goldschmidt suggested.

A miniature wing is extremely similar to a wild-type wing, apart from the reduction in scale, but there is some relative distortion at the proximal region. The insertion of the wing on to the body is not much smaller than normal, and one therefore finds that the basal part of the wing is more nearly normal in its size than is the more distal region. This causes a reduction in the angle (θ) between the directions of L 2 and L 5, which may be no more than 22–23°.

Other genes which affect the wing as a whole are lanceolate (*ll*), narrow (*nw*), broad (*br*), and expanded (*ex*) (Text-fig. 15). The first two, as their names imply, produce long narrow wings, while the last two produce broader, shorter ones. In all cases the deformation is a general one, since the veins appear to fit quite naturally into the new shape of the wing. Goldschmidt has pointed out that *ex* acts in very early stages, by which he probably means the inflated stage. Broad (Pl. 5, figs. 12, 13) and lanceolate certainly act as early as PP 2. The action is therefore earlier than that of dumpy, and it seems that we should seek for it an explanation of a different type. The hypotheses which suggest themselves are that the deformation is due to increased cell division in certain directions, the mutual arrangement of the cells remaining unaltered, or that the change in shape of the wing is correlated with a change in shape of the individual cells. It should be possible to choose between these hypotheses by studying the distribution of the hairs of the adult wing, since each of these may be taken as representing a single cell. If the shape of the cells is altered, they will fit together in a different way which will be reflected in the hair distribution.

It has not been easy to decide on a suitable method of comparing hair distribution. The hairs are found in fact to lie in lines which continue for some distance, but eventually branch or join up with each other. If the

lines were quite regular there would be no difficulty in estimating the length of the cells in the directions parallel to the length and breadth of the wing; or if the hairs were quite irregularly distributed there are techniques (neighbourhood analysis) which would enable one to discover the average shape of the cells. Since the distribution is in fact only partly regular, the method which was finally adopted was to choose regions of the wing in which there were regular lines of hairs running in such directions that estimates of length and breadth were easily made (see Text-fig. 7).

Fairly extreme examples of lanceolate-2 and broad were chosen; the ratio of length to breadth of the whole wings was 2·3 for the lanceolate and 1·73 for the broad. However, this difference was not reflected in the arrangement of the cells, since the average length to breadth ratio of hair distribution was $1·99 \pm 0·06$ for lanceolate-2 and $1·895 \pm 0·055$ for broad. There is therefore no significant difference between these two types in cell shape; the difference must be one of cell number, dependent on differential rates of cell multiplication.

One general point might be raised about the mutants considered in this section. It is characteristic of them that in spite of the change in shape the margin of the wing is intact. The wing fold must therefore have occurred along the margin. Now the occurrence of mutants of the scalloped type makes it clear that the position in which the wing fold appears is not determined by the position of the margin, and mutants such as shifted and four-jointed, approximated, etc., show that the veins can be shifted relative to the margin. It is therefore difficult to frame any hypothesis which would allow genes such as *nw* or *br* to act before the folding, since they would then have to affect, in exactly the same way, the three independent processes of folding, vein determination and margin determination. It is much more plausible to suggest that the deformation is produced by changes in growth-rate occurring after the wing has been everted, that is to say not earlier than P 2. The differential growth of broad, lanceolate-2, narrow and expanded in that case would occur during the later part of PP 1 and early in PP 2; and this conclusion seems very reasonable, since this period is known to be a time when rapid growth is proceeding.

Summary

The genes miniature and dusky produce smaller wings by an effect on cell size. This effect is exerted partly at early prepupal stages and partly during the final expansion of the wing at the end of the pupal

period. There is also some distortion of the wing owing to the disproportionate reduction of the distal and proximal parts.

Lanceolate-2, narrow, broad and expanded produce general alterations in the shape of the wing of the kinds indicated in their names. The veins are altered to fit the wing margin. The effect can be detected in the middle stages of the prepupal wings of the two mutants examined, lanceolate-2 and broad. A study of the hair distribution in adult wings shows that the relative arrangement and shape of the cells is not significantly different from normal, so that the changes in shape must be due to effects on rates of cell division in different directions. Since these affect the veins, they must occur after vein determination, and since they can be seen in the prepupal wing they must occur before that time.

<center>LOCALIZED ABSENCES OF VEINS</center>

There are several genes known which produce breakages, or local absences, of veins. The development of some of these has been studied.

Radius incompletus (*ri*) (Pl. 3, fig. 12; Text-fig. 12) is a gene which causes breakages in L 2. It was studied in *D. simulans*, in which there is an allele with a very regular and marked effect. Since the radius is not represented in the prepupal wing, no sign of the mutant type is to be expected there, and none was found; the wings appeared perfectly normal. As has been pointed out in the description of normal development, L 2 appears during stage P 2c by the anastosmosis of small cavities between the basal processes of the cells of the two epithelia. These cavities flow together to form a continuous lumen, which extends from the base of the wing distally, and also extends inwards, more slowly, from the margin to meet the basal part of the vein. In radius incompletus this anastosmosis fails to proceed, so that only the basal part, and a small section near the margin, become the definitive vein. The detailed timing of the process has still to be investigated, and may prove to provide the explanation of the failure of the vein to extend throughout its whole normal course. The effect remains a localized one.

The vein L 3 is attacked by tilt (*tt*) (Pl. 3, figs. 9, 10), which causes a section of the vein to be missing in the adult. In prepupal stages, L 3 is represented by the anterior branch of the central vein. In tilt flies this appears quite normal. Even in the pupal wing, the vein L 3 appears quite normal and unbroken at the earliest stage at which it can be fully made out. Soon after this, however, a section of the vein disappears. It will be remembered that as the two epithelia come together as the wing contracts from the inflated stage, the cells of the intervein regions

put out basal processes which fuse with those of the cells of the other surface; the veins are lines along which this protrusion of basal processes fails to occur. It is clear that in tilt the whole of L 3 at first behaves like vein material, and fails to put out processes, but that later the section of L 3 which is eventually missing fails to retain its vein character, and forms basal processes just as the intervein material does. It is clear that the vein material has the capacity to put out processes, but that normally this capacity is inhibited; while in tilt the inhibition breaks down over the stretch which finally disappears.

Very similar behaviour is shown by veinlet (*ve*) (Text-fig. 12) which removes the distal tips of L 3, L 4 and L 5. Again the prepupal venation
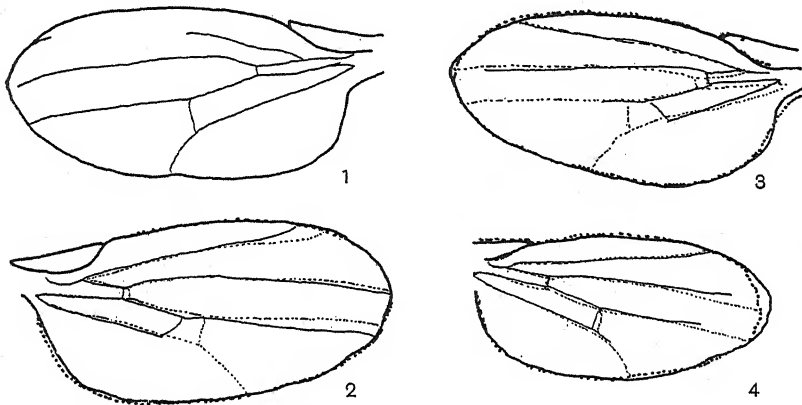
Text-fig. 12. Adult wings. 1, radius incompletus (*D. simulans*); 2, abrupt-2 superposed on normal; 3, veinlet on normal (note shortness of L 5 and position of posterior cross-vein); 4, veinlet on normal, L 5 longer and cross-vein in normal position.

is perfectly normal; and again the veins, at least L 3 and L 4, are formed normally in the very earliest stages of their appearance in the pupal wing (Pl. 3, fig. 11). They disappear in exactly the same way as in tilt, by the appearance of basal processes from the vein cells which should normally not form them. By the time these processes appear, the differentiation of the veins has already proceeded some distance, and the cells which should become vein surface have aggregated to a fairly high dense epithelium. When this differentiation breaks down, and these cells begin to put out basal processes which obliterate the lumen of the vein, the strands of thickened epithelium can still be recognized, in sections, extending beyond the vein lumen over the region from which the vein has disappeared. In a short time, however, they become indistinguishable from the rest of the wing surface.

The effect of veinlet is not entirely confined to a removal of the distal tips of the longitudinal veins. In wings in which a large part of the end of L 5 is lost, it is found that the posterior cross-vein runs at an abnormal angle; instead of meeting L 4 nearly perpendicularly, it makes with it an angle which is quite acute on the proximal side. Moreover, in wings in which so much of L 5 is missing that the remainder does not reach the point where it would normally join the posterior cross-vein, it is found that the cross-vein is shifted proximally. This effect on the cross-vein is discussed more fully later (p. 132).

Cubitus interruptus (ci) (Pl. 3, figs. 5–8) is another gene which removes part of the venation. The allelomorph ci usually causes the absence of the proximal part of L 5 and the distal part of L 4, but in ci^W the proximal effect is usually on L 4 instead of L 5. This lack of specificity is rather remarkable, but it is probable that the proximal ends of L 4
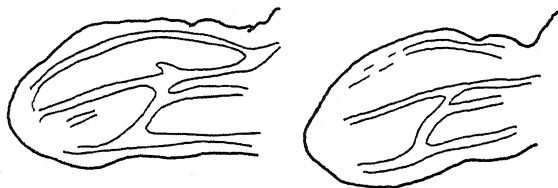
Text-fig. 13. Two wings of cubitus interruptus, stage PP 3, showing prepupal venation Note absence of lower branch of main vein, and junction with posterior vein.

and L 5 both derive from the posterior prepupal vein, so that a disturbance in the development of that vein might lead to either result.

Cubitus interruptus is the only one of the genes causing vein breakage which has visible effects in the prepupal period (Text-fig. 13). The posterior branch of the main prepupal vein is always badly formed and often completely absent; and there is always some sign of a junction between the main vein and the posterior one and in some cases there is a definite lacuna connecting them. The prepupal veins, in fact, may have much the same arrangement as those seen in an adult ci^W, except for the absence of L 2; actually there are probably always slight traces of the missing veins, since even when these cannot be definitely seen in the prepupal wings, they appear in the pupal stage, probably from traces which would not be easy to see in the earlier stage.

The frequent presence of traces of the missing veins makes it impossible to consider ci as determining a general deformation of the vein system comparable to that produced by shf or the fj, d, app group. Cubitus interruptus must act by partially destroying already formed veins. But, as we have seen, it certainly does so during the prepupal

period. Correlated with this early activity, the wings show more evidence of regulatory phenomena than are found in those mutants in which the veins are destroyed in the pupal period. Thus we find in ci^W a very regular occurrence of a smooth connexion between L 3 and the tip of L 5, which can presumably be regarded as constructed out of the cross-veins together with part of L 4. Again, if a ci wing is superposed on a wild type, it will be seen that the end of L 5 is moved distally, as though to compensate for the lack of L 4 (Text-fig. 14). This shift is in exactly
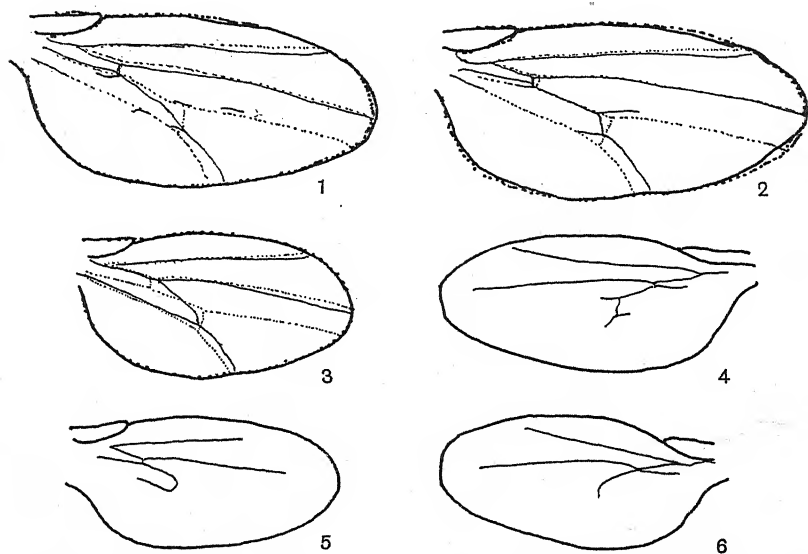
Text-fig. 14. Adult wings. 1 and 2, cubitus interruptus superposed on normal; 3, cubitus interruptus-Wallace on normal; 4, 5 and 6, veinlet-cubitus interruptus. Note position of posterior cross-veins.

the opposite sense to the splaying produced by fj, etc. In the compound containing fj and ci, the end of L 5 is moved distally relative to that of a simple fj, and thereby is brought back again to almost the same position as it has in wild type (Text-fig. 11). The fj and ci effects are therefore additive, in contrast to the fj and app effects for instance. This suggests that whereas fj, d and app all act to produce the same definite pattern, ci acts in some different way. This is consistent with the suggestion advanced above that the former genes act before the eversion of the wing, while ci acts in the early prepupa after the veins have been formed.

The early action of ci, as compared with ve, for instance, also provides an explanation for the very different behaviour of these genes when combined with plexus. The latter is a gene which causes the appearance

of extra veins, and appears to act in the pupal period (see p. 130), that is, at about the same time as *ve*, and after *ci*. In compounds with *ci*, its action can be easily seen; the extra veins are formed around the characteristic *ci* venation, much as in simple *px* wings they are formed around the normal venation (cf. Text-fig. 11). On the other hand, in the F 2 of a cross between *ve h th* and *fj px sp* all the flies containing *h* and *fj sp* had normal wings. It appears that the vein-destructive effect of *ve* had been cancelled by the tendency produced by *px* for extra veins to be formed.

Summary

Of the genes causing breakages of veins, radius incompletus (in *D. simulans*) acts on the special process by which L 2 is formed; the small cavities between the bases of the epithelia fail to fuse together in the normal way.

In tilt, which causes the disappearance of a section of L 3, and veinlet, which attacks the distal ends of the veins, the prepupal venation is normal, and so are the very first beginnings of the pupal veins, but the vein type of differentiation fails to persist in the regions mentioned; the cells, although they at first condense to a fairly high epithelium with no basal processes, finally put out basal processes and become like the intervein cells.

In cubitus interruptus the effect (a disappearance of the distal end of L 4 and of the proximal end either of L 4 or L 5) can already be seen in the prepupal venation. It is presumably exerted during the prepupal period in a manner similar to that in which veinlet and tilt affect the pupal development. Correlated with the early action, there is considerable regulation of the vein pattern, notably a smoothing out of the connexion between L 4 and L 5 in ci^W.

Cubitus interruptus behaves simply additively with plexus, a gene which acts during the pupal period to produce extra venation, whereas when plexus is combined with veinlet, which acts during the same period to cause loss of vein material, the two actions seem to cancel one another out.

THE FORMATION OF EXTRA OR SWOLLEN VEINS

Several mutants are known which cause cells which should normally differentiate as wing membrane to become vein. They may form extra veins, either attached to the normal veins or free from them, or they may merely add to the thickness of the normal veins. The genes of this type

which have been investigated are net, Delta-6, plexus, blistered-2, balloon, bloated and Wrinkled.

In all of these the venation of the adult wing, although abnormal, has the same fundamental pattern as that of wild type. The abnormalities appear as fluctuating variations around the normal condition. This alone is enough to make one suspect that the genes have not altered the basic pattern of the veins as they are determined before eversion, but have altered the conditions under which those partially determined veins have later differentiated. This suspicion is borne out by the facts of development. In all the mutants (with the possible exception of bloated, see below) the prepupal venation is perfectly normal. The abnormalities first appear during the formation of the definitive veins in P 2.

In Delta-6 the effect is primarily a thickening of the veins, particularly along L 2 and the distal end of L 5 and at places at which two veins join (Pl. 5, figs. 14–16). The name of the mutant is in fact taken from the triangular thickenings formed at the junctions of L 2, L 3 and to a lesser extent of L 4 with the margin. One can see these thickenings developing during stage P 2. In normal development the veins are at first rather broad and gradually become narrower during this stage, and it appears that in *Dl*-6 this does not occur normally. However, the cells of the vein-surfaces become condensed to form thick epithelia which stain darkly, so that the mutant effect is not merely an inhibition of the narrowing of the veins. No further analysis of it has been possible.

In balloon the adult wing may show blisters filled with body fluid. In the stock used in this investigation, however, the effect was a different one; there was a slightly increased deposition of chitin over much of the intervein membrane, particularly between L 2 and L 3 and between L 4 and L 5 (Pl. 5, figs. 4–6). The distribution of this chitin was rather irregular, forming patches which were hardly definite enough to be considered as extra veins, but which were histologically intermediate between veins and the normal membrane. In development the first signs of the abnormality can be seen in the latter part of stage P 2, when the wing epithelia are condensing and losing their spongy appearance. It is then found that in balloon wings the spongy texture of the above-mentioned regions is not lost but becomes coarser. The epithelia form patches of properly condensed material, scattered between which are lighter areas in which there is still a space between the bases of the cells. On examination of whole mounts under a high power, one can usually see one of the large phagocytic blood cells occupying, but not filling, each of these spaces. One must conclude that during the contraction from the

inflated stage in balloon phagocytes become trapped between the basal processes of the cells as the membranes close together; whether this is because there is a higher concentration of phagocytes in the body fluid of balloon, or whether it is for some other reason, cannot yet be said. The entrapped blood cells clearly prevent the withdrawal into the cells of the basal processes in their neighbourhood, and keep open a certain space around themselves. They thus create conditions which resemble those in a vein in so far as there is a space, presumably filled with body fluid, underneath the superficial epithelium. The fact that the epithelium above such spaces becomes chitinized indicates that these conditions are among those which are causally effective in the development of the normal vein surface.

An exaggeration of the conditions seen in balloon is found in bloated (Pl. 5, figs. 10, 11). The wings here are completely irregular, with thick but weakly developed veins, and do not entirely lose their pupal folding. The most interesting feature of them is the presence of small spherical vesicles, formed of typical wing membrane with hairs, lying within the thickness of the wing between the upper and lower surfaces. The wing is also smaller than normal, with smaller cells, and has an oblong, blunt-ended shape. In development it is normal in size and shape during the prepupal period, although it may be that the veins are already slightly thicker than usual. The abnormality first becomes noticeable as a profound inhibition of the contraction in P 2. The texture remains spongy, and the veins broad with irregular swellings. Within the spongy intervein material quite large droplets of fluid can be seen. Sections show that surrounding each drop is an epithelium, the cells of which are fairly thin and tightly stretched. It is clear that during the contraction the body fluid is not properly squeezed out of the inflated wing but that droplets remain among the basal processes. Further, it appears that cells migrate in from the epithelia and enclose the droplets; it is interesting to find that they can still differentiate into typical wing epithelium even in this abnormal position. The square-ended shape of the wing, which is similar to that of the inflated stage, can be regarded as a failure of the contraction. Here again one would wish to know whether the abnormal contraction is due to an increased amount of body fluid or to a weakening of the contractile force of the epithelium. The fact that the abnormality also affects the narrowing of the veins would perhaps support the first of these suggestions. Moreover during the dissection of the pupae an impression was gained that they were abnormally "juicy"; but no quantitative data are yet available.

The gene Wrinkled (Pl. 5, figs. 7–9) perhaps hardly belongs in this category, but its effects are somewhat allied to those of bloated, in that they are concerned with the internal fluid pressure, although they occur at a different time, and through a different mechanism. In the adult the wing is fairly normal except that it remains more or less folded up as in the late pupa. During development, no abnormality of the wing blade is apparent. But if one looks at the base of the wing, where it narrows down to become attached to the body, it is found that the wing insertion begins to form even in the prepupal stage, and at any age is always much narrower than in comparable normal wings. It is also abnormally small in the adult wing. Probably the failure of the expansion after emergence from the pupa is due to the constriction of the veins as they pass from the body into the wing, which would interfere with the flow of body fluid necessary to unfold and expand the wing.

The three genes net (Pl. 3, figs. 1–4), blistered-2 (Pl. 5, figs. 1–3) and plexus are closely allied to one another and to balloon. Blistered-2 and plexus, in fact, differ from balloon mainly in that the spaces which remain within the intervein areas are larger and give rise to more definitely chitinized veins in the adult. The development suggests that the comparatively large size of the spaces is due to a coalescence of smaller spaces, like that which occurs normally during the formation of L 2; and it may be significant that the regions on both sides of L 2 are particularly prone to form extra veins, although it must be pointed out that extras are also usually formed in the area between the ends of L 4 and L 5.

In plexus, and to a still greater degree in net, the balloon-process is accompanied by another, which consists of a considerable thickening of certain regions of the wing, which appears somewhat similar to the phenomena seen in Delta. In net the whole space between L 4 and L 5, throughout their length, becomes condensed to a coherent epithelium at a time when most of the rest of the wing is still spongy. At the margin the thickening proceeds further, so that in sections the cells form a very high, closely packed epithelium. The same thing occurs at the distal end of L 2, between it and L 3. In the later stages of P 2, a vein lacuna has usually appeared along the inner edge of the thickening. In the adult, these veins can usually be recognized; one runs from the end of L 2 to join L 3 more or less at right angles, while one runs parallel to the margin in the space between the distal ends of L 4 and L 5. The thickening at the end of L 2 usually seems to become evened out and to form normal wing membrane, distal to the extra cross-vein just mentioned, but the thickening between L 4 and L 5 often persists as a vein-like, heavily

chitinized area. These extra veins are, of course, additional to the extras produced by the balloon-like process. No reason for these additional phenomena in net can yet be given.

One result of the exaggerated condensation of the above-mentioned regions in net is a stretching of the distal part of the area between L 3 and L 4. This is a purely mechanical stretching, as can be seen from the size of the cells. They are 40 % larger than the unstretched cells near the anterior cross-vein. In the same way, the posterior cross-vein is pulled distally, and the space enclosed by it and the proximal parts of L 4 and L 5 becomes long and narrow. By estimating the area of this region, and the area of the cells, one can get a figure for the number of cells contained in it; it comes out at about 95 % of the number contained in the same area in normal wings, which is as near as the measurements could be expected to agree. The deformation of this region is therefore a mechanical consequence of the condensation of so much of the normally epithelial material to form extra veins.

Summary

It appears that if, at the time of chitin formation, there is still a cavity at the base of the cells in a region of the epithelium, those cells will deposit extra chitin and become vein-like. This occurs in balloon, where the persistence of the cavities is due to the trapping of blood cells among the basal processes during the contraction from the inflated stage.

In net, plexus and blistered-2 similar events occur, but the cavities for some unknown reason have a greater tendency to fuse together to form definite extra veins. Particularly in net, there is also a great thickening of the margin, where the epithelium becomes very thick and solid; veins are usually formed along the inner edges of the thickened region, although the whole of it may become vein-like. There is a similar thickening of the margin, and also a thickening of the vein-junctions, in Delta-6, which is probably accompanied by a partial failure of the normal contraction.

The increased condensation of parts of the margin in net and of the material near the veins in Delta may mechanically stretch other parts of the wing.

In bloated droplets of fluid may be trapped in the wing during the contraction. They become clothed with epithelium which differentiates into typical wing membrane with hairs, and they also profoundly disturb all the subsequent morphogenetic processes.

In Wrinkled the final effect is an incomplete expansion of the wing after emergence from the pupa. This is due to the narrowness of the wing

insertion, which in Wrinkled starts to contract prematurely, in the pre-pupal stage, and eventually becomes so small that it constricts the veins.

THE POSTERIOR CROSS-VEIN

The posterior cross-vein is the last of the veins, except sometimes for L 2, to appear during the contraction. It is simply the last trace of the central cavity of the wing-sac. In cross-veinless *cv* this cavity closes up without leaving any remnant which serves as a cross-vein; so far no antecedents of a possibly causal nature have been discovered in con-nexion with this (Pl. 4, figs. 12, 13).

In several of the mutants which have been discussed, it has been remarked that the position of the cross-vein has been shifted. This was true in net, where the shift was due merely to a mechanical distortion of the wing consequent on the supercontraction of the posterior margin between the ends of L 4 and L 5. Similarly, the shift which occurs in dumpy is mainly due to a mechanical distortion occurring after the formation of the vein, although in this case the evidence may indicate a slight sliding of the cross-vein relative to L 4. In *d, app* and *fj*, on the other hand, the shifting of the cross-vein can certainly not be attributed to mechanical distortion and therefore involves the production of the vein by cells which in a normal wing would be part of the membrane and not part of a vein surface. That is to say, whereas in the other cases there has merely been a change in the position of the cross-vein owing to stretching, here there has been a change in the place at which the vein is determined. This alteration in the position of determination is certainly correlated with the general deformation and splaying out of the longi-tudinal veins. The question therefore arises whether the position of the cross-vein is determined at the same time as that of the longitudinal veins and is correlated with the latter in a "field" manner, or whether the determination of the cross-vein occurs later and is secondarily affected by the conformation of the longitudinal veins.

Critical evidence as to this point is given by veinlet. In this mutant the prepupal wing is normal, and the tips of the veins can even be seen in the earliest stage of the contraction; the vein-disappearance occurs soon after this, in P 2*b* and P 2*c*. Now if L 5 remains fairly long in a *ve* wing, the posterior cross-vein may occur in its normal position; but where L 5 is considerably reduced in length, the cross-vein appears at the end of the remnant of L 5, and that may involve its being shifted proximally (Text-fig. 12). The same phenomenon occurs in abrupt, but we have no knowledge of when the vein destruction occurs in that type.

The behaviour in veinlet, however, clearly shows that events occurring in early P 2 can decide which cells shall form the cross-vein. Its final determination must therefore occur at about the time of its appearance and long after the determination of the longitudinal veins. It might be possible to argue, and some of the appearances in *ci* wings might support such a suggestion, that there is some kind of preliminary "Bahnung" (in Vogt's sense) of the cross-vein during the prepupal period. But a discussion of this would be involved and would be unlikely to lead to any clear conclusions.

It is worth pointing out the different shapes and angles assumed by the cross-vein according to what other veins it is attached to (Text-fig. 14). All the veins in this region behave in a way which is formally analogous
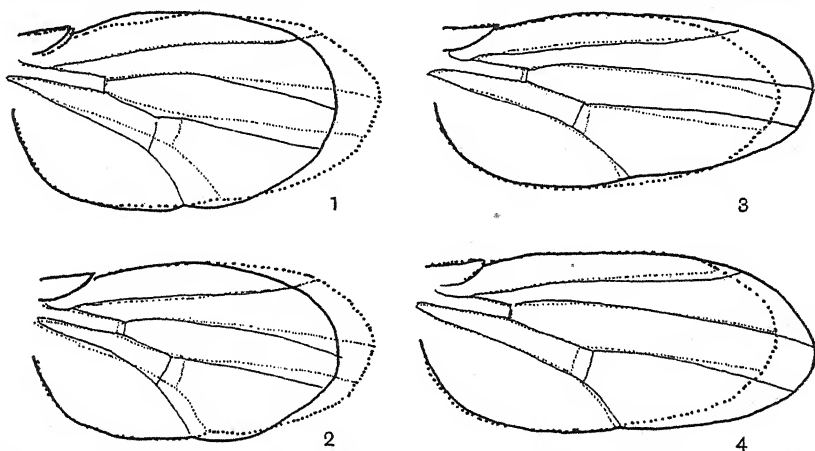
Text-fig. 15. Adult wings. 1, broad superposed on normal; 2, expanded on normal; 3, lanceolate-2 on normal; 4, narrow on normal.

to the behaviour of elastic threads which are under some tension and are capable of sliding along one another. The sliding inwards of the cross-vein when L 4 and L 5 are bent apart by *app*, etc., raises the same train of thought. One is reminded of D'Arcy Thompson's attempt to analyse some animal patterns in terms of soap-bubble models. Perhaps the formal analogy is not so deceptive; the veins are originally hollow spaces within the epithelium, and they may not be so far from soap bubbles in their mechanical properties.

Summary

The position of the posterior cross-vein is dependent on the positions of the longitudinal veins in its neighbourhood. It behaves like an elastic thread connected to L 4 and L 5 but able to slide along them. Its

position is affected by events occurring in the longitudinal veins as late as the pupal period; for instance, by the disappearance of the end of L 5 in veinlet wings. The determination of the vein therefore cannot occur earlier than this time; it must in fact occur almost at the time of the definitive appearance of the vein.

THE CURVATURE OF THE WING

The gene Curly causes an upward curving of the wings, while curved has the effect of causing them to curve downwards. In the development of these wings up to the end of stage P 2 d, no abnormality of any kind could be detected. It seems highly probable, therefore, that there is no visible action of these genes until the time when the wing of the newly emerged fly is drying out to its final adult form. The curvatures could then be produced by slight differences in the contraction of the upper and lower surfaces. This interpretation is supported by the well-known fact that Curly, and to a less extent curved, are extremely dependent on environmental conditions for their grade of expression.

GENERAL SUMMARY

This paper gives an account of the development of the wing of *Drosophila melonogaster*, both in the wild type and in the following mutants: scalloped, vestigial, vestigial-nipped, Beadex-J, Beadex-C, Beadex-3, Lyra, cut-6, Xasta, dumpy, dumpy-02, Blade (of *D. pseudo-obscura*), four-jointed, shifted-2, veinlet, tilt, radius incompletus (of *D. simulans*), cubitus interruptus, cubitus interruptus-W, plexus, net, blistered-2, balloon, bloated, Delta-6, broad, lanceolate-2, miniature, dusky, Wrinkled, curved, Curly. The following mutants were studied only as adults: spade, approximated, dachs, narrow, expanded.

These thirty-eight genes affect sixteen different, but not necessarily independent processes which occur during wing development. These processes may be listed, approximately in chronological order, as follows:

(1) Before the wing is everted, it consists of a thickened region of the surface of the mesothoracic imaginal buds. Each bud is an ovoid sac, and the wing region is on the posterior part of the ventral surface; it will eventually be folded into the sac, and then pushed out through the dorsal surface as a flat plate, which is composed of two epithelial layers folded together. Before this folding occurs, a pattern of longitudinal veins is determined on the future wing surface at some time during the late larval period. The process is influenced by shifted-2, which causes the veins to diverge from one another at a smaller angle than normal. The

genes four-jointed, dachs and approximated cause an increase in the angle between the veins and possibly act at this time.

(2) The fold by which the wing region is pushed into the bud and thus everted eventually becomes the wing margin. Normally it coincides with a line which is determined to develop the marginal vein and bristles. But the position of the fold is affected by the "scalloping" genes (scalloped, vestigial, vestigial-nicked, Beadex-J, Beadex-C, Beadex-3, Lyra, cut-6, and Xasta). These shift the fold in relation to the determined margin, so that some of the margin is missing from the adult wing.

(3) As soon as the wing region has folded together, the veins of the prepupal stage can be seen as hollow tubes between the two surfaces. Their development is affected by cubitus interruptus and cubitus interruptus-Wallace, which cause the disappearance of certain regions and the fusion of others.

(4) At the same time the wing expands, both by cell division and cell expansion. The relative rates of cell division in different directions are affected by broad, expanded, lanceolate-2 and narrow, of which the first two cause the wing to be relatively broader and the last two relatively longer. The expansion of the cells is partly inhibited by miniature and dusky.

(5) The base of the wing does not normally begin constricting so as to form the wing-insertion until some time later, but in Wrinkled flies the process begins at this time.

(6) About 9 or 10 hr. after puparium formation the wing becomes highly inflated by internal pressure, which forces the two surfaces apart. The height of this inflation corresponds with the beginning of the true pupal period, during which the wing contracts again to a flat plate. There are four main factors concerned in this contraction.

(*a*) The contractility of the wing epithelia. This is relatively increased in dumpy, dumpy-02, Blade (of *D. pseudo-obscura*) and probably in spade. The resulting deformation may be a shortening of the wing, as in dumpy and spade and occasionally in Blade, or an elongation as is more usual in Blade.

(*b*) The veins. Possibly different vein patterns have an effect on the contraction, but it is slight (cf. dachs etc.).

(*c*) The general shape of the wing. In scalloped wings with a generally elongated shape the elongation is increased, in those with a rounded shape the breadth is increased.

(*d*) The expressing out of the wing of the body-fluid and its contained cells. In balloon, blood cells become caught among the processes which

extend between the two epithelia. In bloated, droplets of fluid remain within the cavity of the wing and become clothed with cells which should belong in the epithelia.

(7) As the wing contracts and the two epithelia close together, the definitive veins appear as lacunae. The distal tips of these fail to persist in veinlet, and the middle portion of the third longitudinal vein (the medius) disappears in tilt; abrupt, which removes the end of the fifth vein, probably acts at this stage.

(8) The radius, or second vein, is formed in a different way to the other longitudinal veins by the coalescence of small spaces. This process is inhibited by radius incompletus (of *D. simulans*).

(9) The posterior cross-vein is formed as the last trace of the central cavity of the wing. This does not persist in cross-veinless. The position of the cross-vein is not determined at the same time as that of the longitudinal veins, since it is dependent on the relations between the longitudinal veins in its neighbourhood, and these may be altered, by genes such as veinlet, for instance, as late as the pupal period.

(10) After the wing has attained the form of a flat plate, the veins continue to become narrower. This is inhibited by Delta-6.

(11) The intervein material develops as wing membrane. This is secondarily affected by balloon, in consequence of the cavities formed around entrapped blood cells, which develop abnormally thick chitin. It is also affected by plexus, net and blistered-2, which cause the persistence, sometimes followed by coalescence, of cavities within the intervein spaces, and thus lead to the deposition of chitin and the formation of extra veins.

(12) The margin becomes thicker, particularly along the anterior edge. This process is increased unduly by Delta-6 and net.

(13) The formation of extra veins entails a greater contraction of these cells than would occur if they developed as membrane. This may cause the stretching of the remainder of the membrane, a phenomenon which is most noticeable in net.

(14) The wing finally expands in area by enlargement of its cells. This process is to some extent inhibited by miniature, dusky and bloated.

(15) After emergence from the pupa, the wing, which has become folded owing to the above-mentioned expansion, is unfolded and stretched by the pressure of the internal fluid. This is partially prevented in Wrinkled by the narrowness of the wing-insertion consequent on its precocious and exaggerated development.

(16) The wing dries out to a thin flat plate. This is affected by Curly,

which causes an undue contraction of the upper surface, and by curved, which has a similar effect on the lower surface.

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EXPLANATION OF PLATES 2—5

PLATE 2

The development of wild-type wings

Fig. 1. Imaginal bud, stage PP 1, age 3 hr.

Fig. 2. Stage PP 2, age $5\frac{1}{4}$ hr.

Fig. 3. Stage PP 3, early, age $6\frac{1}{4}$ hr.

Fig. 4. Stage PP 2, middle, age $6\frac{1}{4}$ hr.

Fig. 5. Stage PP 2, end, age $7\frac{1}{2}$ hr.

Fig. 6. Stage PP 4, early, age 10 hr.

Fig. 7. Stage PP 4, end, age 13 hr.

Fig. 8. Stage P 1, age $17\frac{1}{2}$ hr.

Fig. 9. Surface view of epithelium in stage P 1 (at higher magnification).

Fig. 10. Stage P 2*b*, age $19\frac{1}{2}$ hr.

Fig. 11. Stage P 2*c*, L 2 missing but other veins formed, age $21\frac{1}{4}$ hr.

Fig. 12. Stage P 2*c*, L 2 forming but central vesicle still present, aged $22\frac{1}{2}$ hr.

Fig. 13. Stage P 2*c*, all veins present, age 27 hr.

Fig. 14. Stage P 2*d*, beginning, age $28\frac{1}{2}$ hr.

Fig. 15. Stage P 2*d*, end, age 43 hr.

Fig. 16. Stage P 3, age about 55 hr.

PLATE 3

Fig. 1. *net*, stage PP 3.
Fig. 2. *net*, stage P 2*b*.
Fig. 3. *net*, stage P 2*c*.
Fig. 4. *net*, adult.
Fig. 5. *cubitus interruptus*, stage PP 3.
Fig. 6. *ci*, early in stage P 2*c*.
Fig. 7. *ci*, stage P 2*d*.
Fig. 8. *ci^W*, stage P 2*d*.
Fig. 9. *tilt*, stage P 2*c*.
Fig. 10. *tt*, stage P 2*d*.
Fig. 11. *veinlet*, stage P 2*b*.
Fig. 12. *radius incompletus* (*D. simulans*), stage P 2*c*.
Fig. 13. *leg*, stage PP 2.
Fig. 14. Legs of right side, stage PP 3.
Fig. 15. Tarsal portion of leg at beginning of stage P 1.
Fig. 16. Tarsal portion of leg, stage P 2*a*.
Fig. 17. Leg at stage P 2*c*.
Fig. 18. Leg at stage P 2*d*.

PLATE 4

Fig. 1. *Xasta*, imaginal bud, stage PP 1, note the slight notch at the tip of the wing fold.
Fig. 2. *Xa*, stage PP 3, note deep distal notch.
Fig. 3. *Xa*, stage P 1, slight distal notch.
Fig. 4. *Xa*, stage P 2*d*, the wing is similar in shape to that of the adult.
Fig. 5. *Blade* (*of D. pseudo-obscura*), stage P 1, note rounded shape.
Fig. 6. *Bl*, stage P 2*b*.
Fig. 7. *Bl*, stage P 2*c*.
Fig. 8. *Bl*, stage P 2*d*.
Fig. 9. *cut*-6, stage PP 2, note narrow shape (cf. Pl. 2, fig. 2).
Fig. 10. *ct^6*, stage PP 3, late.
Fig. 11. *ct^6*, stage P 1.
Fig. 12. *ct^6*, stage P 2*b*.
Fig. 13. *ct^6*, stage P 2*d*.
Fig. 14. *vestigial*, imaginal bud, stage PP 1, note small wing fold.
Fig. 15. *vg*, stage PP 4.
Fig. 16. *vg*, stage P 2*a*.
Fig. 17. *vg*, stage P 2*d*, to show contraction of wing inside the sheath.
Fig. 18. *Beadex-J*, imaginal bud, stage PP 1, note small narrow wing fold.

PLATE 5

Fig. 1. *blistered*-2, stage P 2*d*.
Fig. 2. *bs^2*, beginning of stage P 3.
Fig. 3. *bs^2*, adult.
Fig. 4. *balloon*, end of stage P 2*c*.
Fig. 5. *ba*, end of stage P 2*d*.
Fig. 6. *ba*, adult.
Fig. 7. *Wrinkled*, stage PP 2, note narrowing at base of wing.
Fig. 8. *W*, stage P 2*c*.
Fig. 9. *W*, adult.

Fig. 10. *bloated*, stage P 2*d*, note small size and droplets of fluid.
Fig. 11. *blo*, adult.
Fig. 12. *broad*, stage PP 3.
Fig. 13. *br*, end of stage P 2*d*.
Fig. 14. *Delta*-6, stage P 2*c*.
Fig. 15. *Dl*⁶, end of stage P 2*d*.
Fig. 16. *Dl*⁶, adult.

All photographs of pupal wings × 100, of adult wings × 50.

PLATE 5
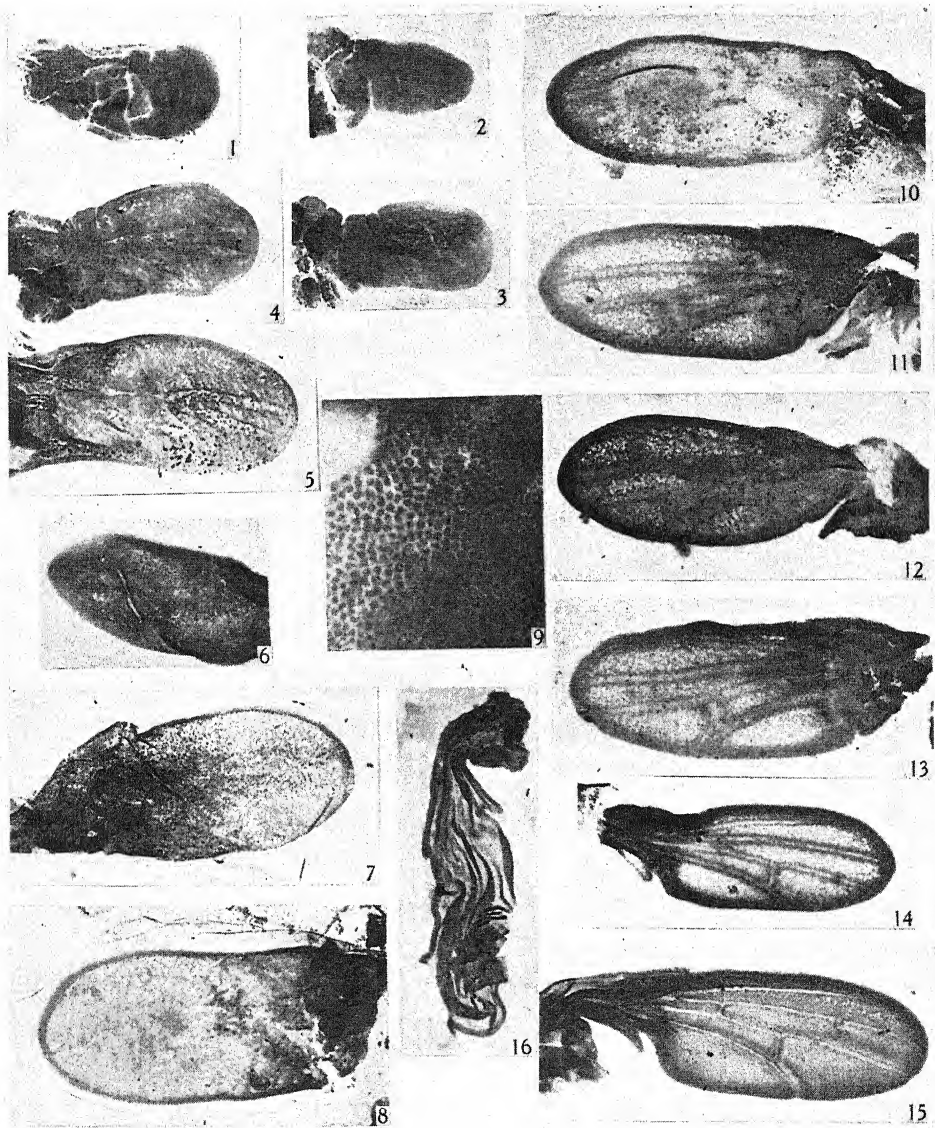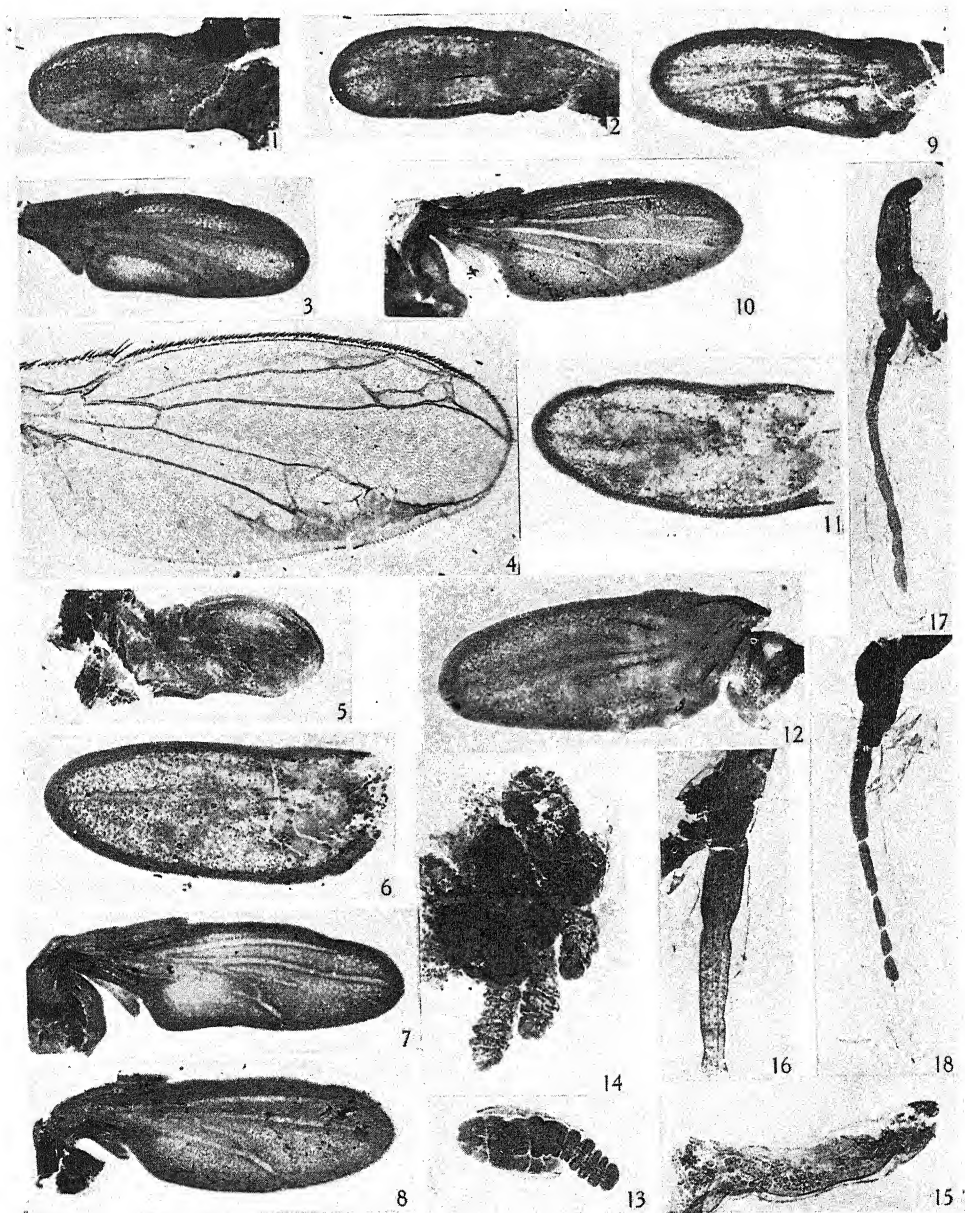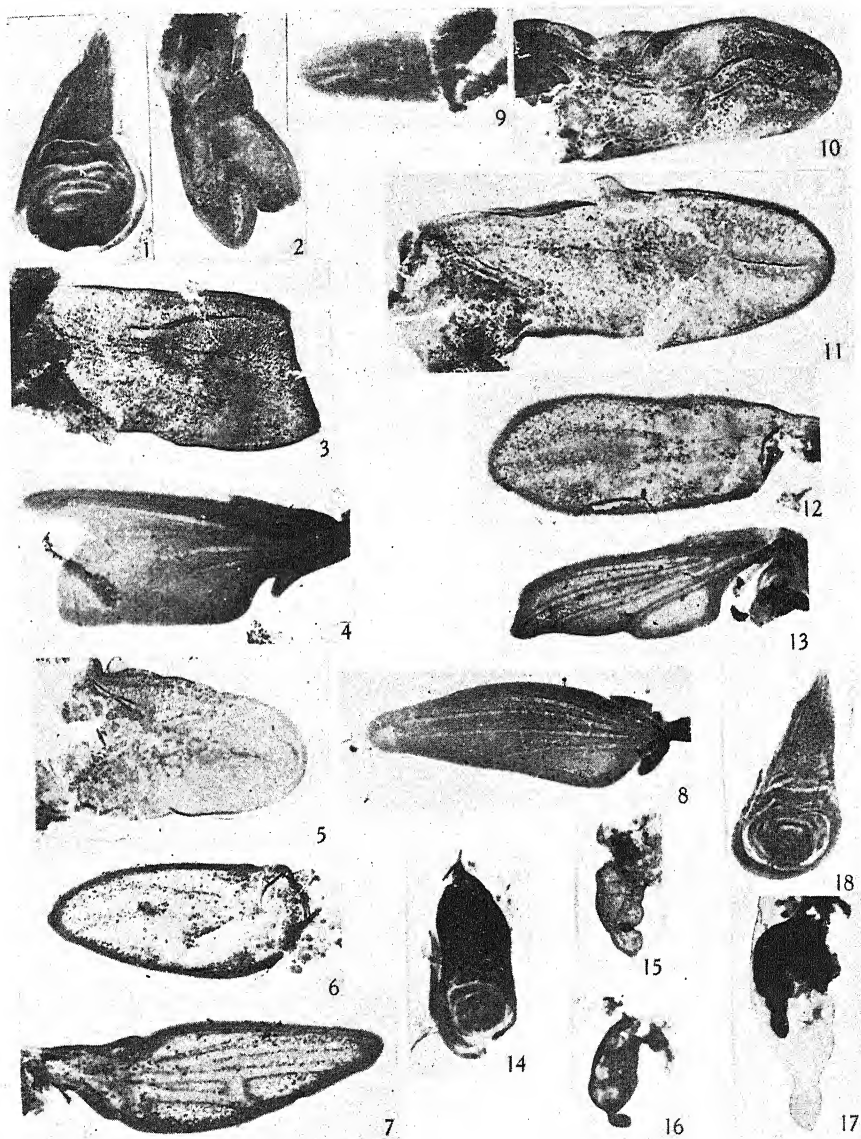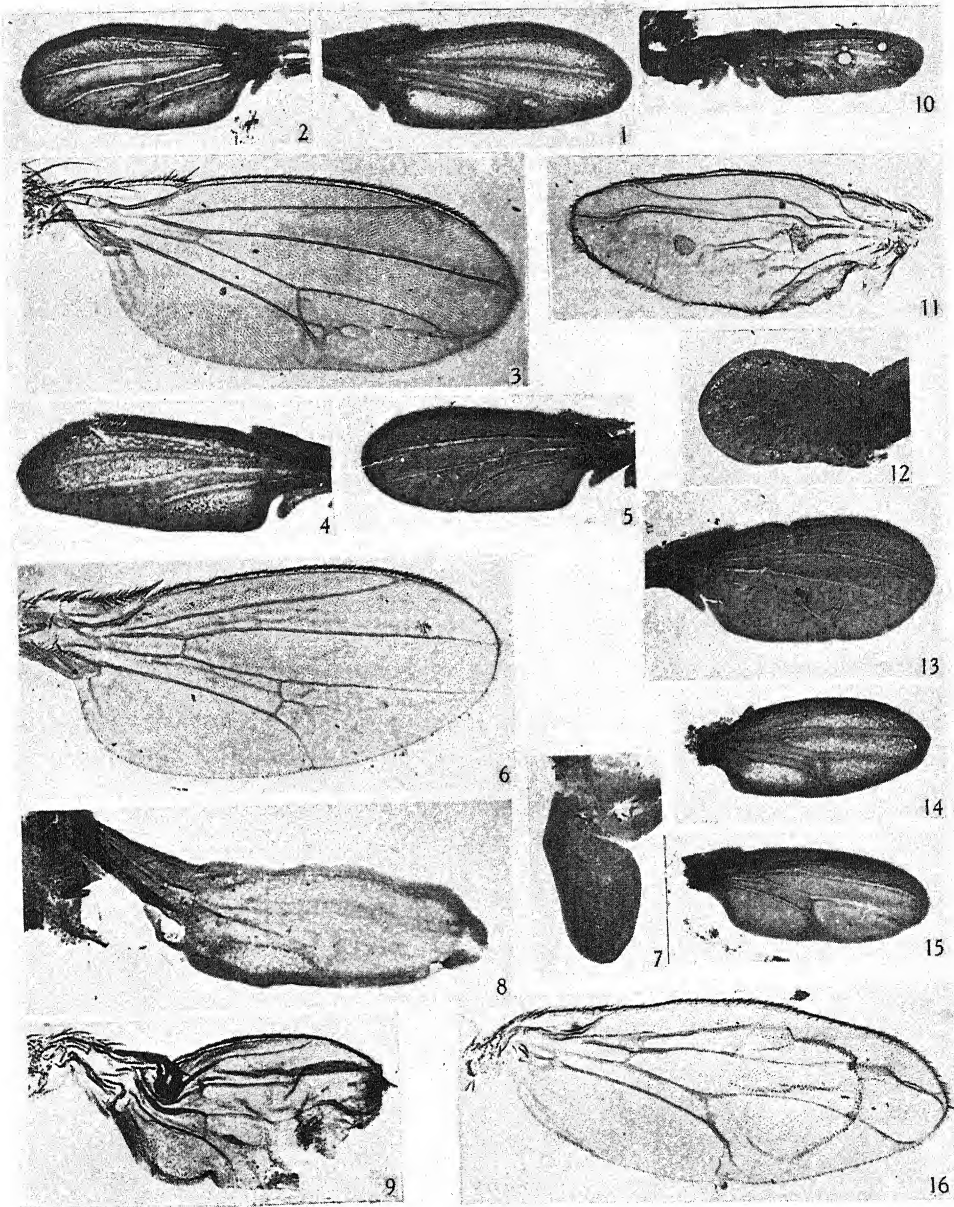
THE PARTIAL SEX-LINKAGE OF RECESSIVE SPASTIC PARAPLEGIA

By J. B. S. HALDANE, F.R.S.

BELL (1939) has collected 273 pedigrees of Friedreich's ataxia, spastic ataxia, and spastic paraplegia in the literature, besides publishing forty-six new pedigrees from her own and Carmichael's observations. From the former group she generally excluded pedigrees including only one case of the disease.

She is inclined to regard Friedreich's ataxia and spastic ataxia as due to the same gene, or genes, perhaps influenced by modifiers. She is more doubtful in the case of spastic paraplegia. For a certain number of pedigrees exist in which some members would be classified as cases of Friedreich's ataxia, others of spastic ataxia. Spastic paraplegia seems to be more sharply defined.

Haldane (1936) reported the presence in man of a group of phenomena which are attributed to partial or incomplete sex-linkage. The genes responsible are thought to be carried in that part of the sex chromosomes which is homologous in the X and Y. Such genes may therefore cross over from one of these chromosomes to the other in a man. At first sight the pedigrees resemble those of autosomal genes. But they differ in the following ways. If a man receives a partially sex-linked gene from his father he hands it down, on an average, to a majority of his sons and a minority of his daughters. If he receives it from his mother he transmits it to a majority of his daughters and a minority of his sons. This can readily be detected in pedigrees of dominants. In the case of recessives it shows up in two ways. Where the parents are related we may assume that they derived the recessive gene from a common ancestor. If the father is related to the mother through his father we expect an excess of affected sons, if through his mother, an excess of affected daughters. If χ be the frequency of recombination between the gene and the differential segments of the sex chromosomes, and if ♂, ♀, ♂̇, ♀̇ represent normal and affected males and females, and a, c, b, d, their numbers, the expectations are:

	♂	♀	♂̇	♀̇
	a	c	b	d
Parents related through father's father	$1+\chi$	$2-\chi$	$1-\chi$	χ
Parents related through father's mother	$2-\chi$	$1+\chi$	χ	$1-\chi$

If the parents are unrelated, or their relationship unknown, Fisher (1936) showed that in a family containing s_1 and s_2 abnormals, the expected value of $u = (a-c-3b+3d)^2 - (a+c+9b+9d)$ is $\frac{1}{9}(1-2\chi)^2k$, where $k = (s_1+9s_2)^2 - (s_1+81s_2)$. And if $\chi = \frac{1}{2}$, the variance of u about its mean value of zero is $2k$. In a large group of families the sampling variance of the sum of the values of u is twice the sum of the values of k. This is true whatever the method of ascertainment, i.e. whether or not there is a bias in favour of families containing large numbers of abnormals.

Bell finds that a majority of the pedigrees of all three diseases are consistent with the condition being due to a recessive gene. Others show a probably dominant type of inheritance. A few show a sex-linked recessive type. In the pedigrees of spastic paraplegia she finds 55% affected in the sibships showing a dominant inheritance and (after suitable correction) 27·1% in those showing a recessive type. The age of onset is, with few exceptions, very close in members of the same sibship. For spastic paraplegia the correlation coefficient is 0·884. Thus we shall make few mistakes if we assume that the elder sibs of affected persons who had not developed the disease at the time of observation would not do so later. On this assumption we can draw up Tables 1 and 2. There is only one case in which the relationship of the parents is given, namely pedigree 562, given in my Fig. 1.
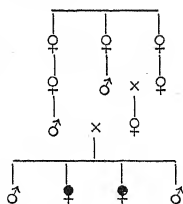
Fig. 1.

The affected daughters had developed symptoms at the ages of 3 and 4. The youngest son was aged 3, and therefore might have developed the disease later, though a clinician generally notes incipient disease before the age of onset. There were thus no cross-overs out of 3 or 4 sibs. The pedigree is consistent with partial sex-linkage, though of course it is of no great significance if taken alone. Table 1 is a summary of those sibships where all sibs except those dying in infancy are recorded. I have counted younger sibs as normal if they had passed the mean age of onset of their affected sibs. Where ages were not recorded I have assumed an interval of three years between sibs. Table 2 is a summary of those where

either the number, sex, or birth order of normal sibs was not recorded. Table 1 gives

$$S(u) = +1022, \quad S(k) = 23{,}570, \quad \sigma = 217{\cdot}1.$$

Table 1. *Sibships with complete information*

Pedigree	♂	♀	♂	♀	$u+$	$u-$	k
280	0	1	0	3	36	—	540
504	1	0	0	2	30	—	198
505	1	0	2	0	6	—	198
507	2	0	4	2	—	40	2648
508*	0	1	7 +1?	0	420	—	3528
509	4	0	2	1	—	30	714
511	1	1	2	0	16	—	236
512	2	0	3	0	20	—	596
513	0	0	1	2	—	18	486
514	3	2	0	2	26	—	362
515	0	0	0	2	18	—	162
515	0	0	2	0	18	—	162
516	1	1	1	1	—	20	236
517	1	0	2	1	—	24	540
518	3	0	1	1	—	12	276
519	1	2	3	1	10	—	1194
520	3	0	1	2	6	—	654
521	0	3	0	4	42	—	1194
524	0	0	2	2	—	36	972
525	0	0	0	3	54	—	486
526	0	2	1	1	—	16	236
527	0	0	0	2	18	—	162
531	1	2	2	0	28	—	276
533	1	2	2	2	—	38	1194
534	0	1	3	0	72	—	540
535	0	0	1	1	—	18	162
536	0	4	3	0	138	—	714
537	3	0	2	0	—	12	276
538	1	0	0	2	30	—	198
556	1	0	3	0	36	—	540
557	1	1	0	2	16	—	236
559	3	0	0	3	114	—	654
563	0	1	4	1	54	—	1710
564	4	0	0	2	78	—	318
566	0	0	1	3	0	0	972
35	38	24	45	48	+1286	−264	23,570

* One pair of twins, possibly monozygotic.

Thus the sum of the values of u is 4·71 times its standard error on the base of sampling, and is undoubtedly significant. If family 508 is omitted, we have

$$S(u) = +602, \quad S(k) = 20{,}042, \quad \sigma = 200{\cdot}2.$$

Thus $S(u)$ is still over three times its standard error, and is, moreover, of the right sign, so that the probability of so large a deviation is 0·0013. In Table 2, u and k are calculated from the affected members only. Including it, we have

$$S(u) = 1130, \quad S(k) = 25{,}514, \quad \sigma = 225{\cdot}9.$$

Thus the sum exceeds zero by five times its standard deviation. There is no doubt at all that some anomaly is really present, and so far no hypothesis other than partial sex-linkage has been put forward which would explain it.

The last set of data give a recombination frequency χ of 18·43 % between the locus of the gene and the differential segments which carry the sex-linked genes. If the two twins in family 508 were dizygotic, we have

$$S(u)=1251, \quad S(k)=26,666, \quad \sigma=230\cdot9, \quad \chi=0\cdot1750.$$

If this family is omitted, we have

$$S(u)=710, \quad S(k)=21,986, \quad \chi=0\cdot2302.$$

However, there seems to be no good reason for omitting this family. It can hardly be explained by ordinary sex-linkage, for the mother had

Table 2. *Sibships with incomplete information*

Pedigree	♂	♀	♂̇	♀̇	$u+$	$u-$	k
499	—	—	0	3	54	—	486
506	—	—	1	1	—	18	162
510	1	1	2	0	18	—	162
522	0	1	0	2	18	—	162
523	4	1	2	0	18	—	162
529	—	—	0	2	18	—	162
530	5	1	2	1	—	18	486
532	—	—	2	0	18	—	162
8	—	—	9	9	144	36	1944

five normal brothers, and Bell states that "This family was very carefully investigated and the presence of the disease excluded in at least 70 members of the stock, in 5 generations; the parents were normal and not consanguineous". However, a standard error for χ can hardly be given in view of the uncertainty introduced by it and the fact that an auto-somal recessive gene may be responsible for some cases. On the other hand, the sampling distribution of $S(u)$ is not seriously asymmetrical when we are dealing with forty-three families, as in this case, and the tests of significance are satisfactory.

We can now proceed to answer three questions. Is a partially sex-linked gene responsible for the dominant type of spastic paraplegia? This would be so if the dominant and recessive forms were allelomorphic, as seems to be the case with retinitis pigmentosa (Haldane, 1936). On examining the pedigrees showing undoubted dominance, in which a generation is never skipped, we find that the children of fathers who derived the disease from their fathers were

$$10 \, ♂, \, 8 \, ♀, \, 13 \, ♂̇, \, 12 \, ♀.$$

The children of fathers who derived it from their mothers were

$$7 \, \male, \ 6 \, \female, \ 6 \, \male, \ 5 \, \female.$$

Thus there were thirty-six affected out of sixty-seven (expected 33·5) and thirty-four cross-overs out of sixty-seven on the hypothesis of partial sex-linkage. There is thus not the faintest suggestion of this phenomenon, or of any other deviation from what is to be expected in the case of an autosomal dominant.

We can also settle the question, raised by Bell, as to whether the same main gene, perhaps modified by other genes or by environment, is responsible for the three diseases, Friedreich's ataxia, spastic ataxia, and spastic paraplegia.

There are six sibships derived from marriages of cousins whose relationship is known. These are summarized in Table 3. FA means that the families showed Friedreich's ataxia, SA denotes spastic ataxia, whilst FA + SA denotes families where both diseases occurred. Very possibly family 371 should be omitted, as the disease occurs in eight sibships in a large pedigree of related stocks. Though it is never transmitted from parent to offspring, we may be dealing with a very irregular dominant. The totals are compared with their expectations, where χ is the recombination frequency:

Obs.	8	2	12	3
Exp.	$\dfrac{10(2-\chi)}{3}$	$\dfrac{10(1+\chi)}{3}$	$15(1-\chi)$	15χ

Table 3. *Offspring of cousin marriages*

Pedigree	Type	$\male$	$\female$	$\male$	$\female$
	(a) Husband related through his father				
422 A	SA	0	0	2	1
463 A	SA	1	1	2	1
		1	1	4	2
	(b) Husband related through his mother				
267	FA	3	0	0	1
328	FA	2	0	0	3
371	FA + SA	2	1	1	2
372	FA + SA	0	0	0	2
		7	1	1	8

The probability of such large deviations from equality in the expected direction is, for the normals 7×2^{-7}, for the affected 9×2^{-9}. Thus the total probability is less than 0·003. This constitutes evidence in favour of partial sex-linkage. However, a search by the method of Tables 1 and 2 gave a negative result. 116 sibships of Friedreich's ataxia gave $S(u) = +48$, $s(k) = 56,728$, $\sigma = 336·8$. Forty-eight sibships segregating for

spastic ataxia gave $S(u) = +162$, $S(k) = 10.05$, $\sigma = 141.8$. Or omitting pedigree 293, where there may be dominance, $S(u) = +170$, $S(k) = 7484$, $\sigma = 122.35$, so that $S(u) = 1.39\,\sigma$. The probability of so large a deviation in the positive direction, in the absence of linkage, is 0.08. Hence there is some reason to suspect the existence of linkage. However, it is very weak compared with that derived from the cousin marriages.

We can say with fair certainty that the gene which is responsible for a large fraction, at least, of the cases of recessive spastic paraplegia, is only responsible for a small fraction, at most, of the cases of Friedreich's ataxia and spastic ataxia. The indications of partial sex-linkage suggest that this gene is responsible for a few cases of these latter diseases, and that by chance two out of the six cases where the relationship of the parents is known were due to this gene. If only about one-quarter of all cases were due to it it would not be detected with certainty where the relationship of parents is unknown. Thus Bell is probably right in claiming some common genetic basis for spastic paraplegia and the other two diseases, but it is very unlikely that all three are due to the same gene. Even if we reject the hypothesis that the large positive value of $S(u)$ found for spastic paraplegia is due to partial sex-linkage, it is a fact which constitutes a difference between the genetics of this disease and those of the ataxias.

Table 4. *Ages of onset in spastic paraplegia*

Pedigree	u	Ages
508	420	1–2, ?, ?, ?, 1–2
521	42	11, 9, 8, 6
525	54	0–4, 0–4, 0–4
534	72	12, 12, 12
536	138	12, 14, 6
559	114	3, 3, 1
563	54	?, c. 37, c. 30, 29, ?
564	78	10, 16

The third question is whether we are dealing with a single partially sex-linked gene, or a series of allelomorphs. In an accompanying paper (Haldane, 1940) I have pointed out that the correlation between ages of onset in sibs is so high that it can only be explained by postulating different genes in different families. Modifiers could not account for it, as they would lead to a considerable variation in the age of onset within a sibship. Bell found the high correlation coefficient of 0.852 for recessive spastic paraplegia. It might be that the partially sex-linked gene gave a fairly uniform age of onset, the outlying cases being accounted for by one or more autosomal recessives. Table 4 shows that this is

not the case. The eight sibships are those with the largest values of u. Indeed, between them they furnish almost all the evidence for linkage. In the first family it is fairly clear that all the brothers developed symptoms in early childhood, though the age is only given in two cases. This table is intelligible if we are dealing with three allelomorphs, causing incidence round the ages of 2, 12, and 30 respectively. Between them they cover almost the whole range of ages of onset. Only five out of 132 cases developed the disease after the age of 40. It is most unlikely that we are dealing with several genes at different loci in the same section of a single chromosome. We are probably concerned with multiple allelomorphs at the same locus, such as certainly occur in the cases of colour-blindness and haemophilia, and possibly in that of retinitis pigmentosa. In accordance with Goldschmidt's theory, we may suppose that all are responsible for fundamentally the same process, but that its rate varies in different members of the series.

SUMMARY

1. Recessive spastic paraplegia is due to a partially sex-linked gene.

2. Dominant spastic paraplegia is due to an autosomal gene. Partially sex-linked recessives are probably responsible for a small fraction of all cases of Friedreich's ataxia and spastic ataxia.

3. There are probably three or more allelomorphs of the partially sex-linked gene, determining different ages of onset.

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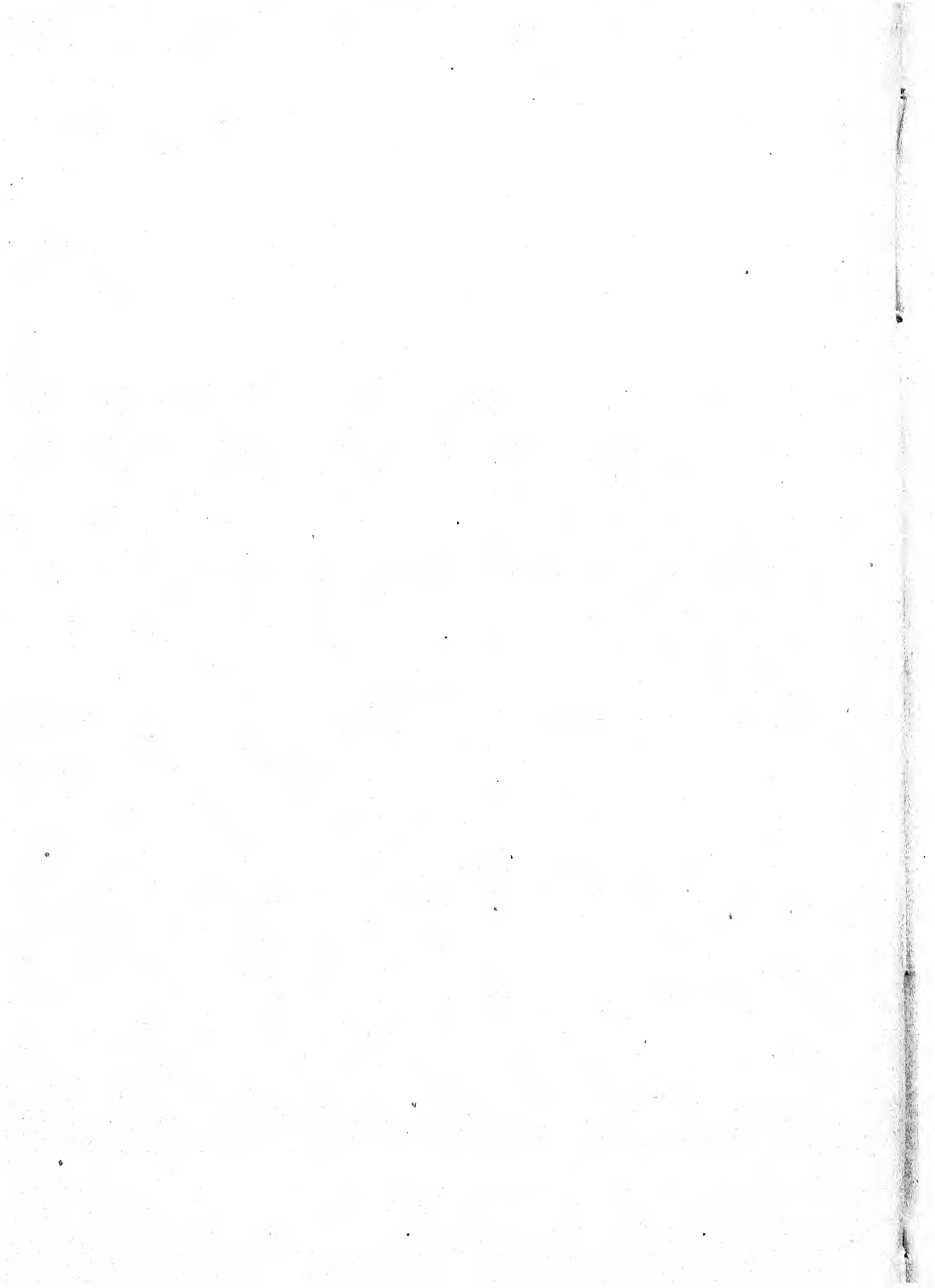
THE RELATIVE IMPORTANCE OF PRINCIPAL AND MODIFYING GENES IN DETERMINING SOME HUMAN DISEASES

By J. B. S. HALDANE, F.R.S.

(With One Text-figure)

A VAST amount of rather speculative theories have been produced regarding natural selection in man. It is probable that in modern civilized communities reproductive selection, that is to say, selection on a basis of fertility, is more important than strictly Darwinian selection on the basis of survival. But the opposite was probably the case through most of man's career. Moreover, what little exact genetical knowledge we possess relates wholly to selection on the basis of survival.

A number of genes are known which diminish the expectation of human life, but yet allow long enough life to permit of reproduction by many individuals. Bell (1939) has shown that in a number of such cases the period between the onset of the disease and death is almost independent of the age of onset. Thus the age of onset is a valuable index of the selective disadvantage of the gene.

With the discovery of modifying genes there has been a tendency in some quarters to postulate their existence and importance on a very wide scale, while other writers have stressed the importance of environment. Among a group of persons affected with a given disease, whose genetical basis is roughly known, we can examine the relative importance in determining the age of onset, of:

(1) Differences in the main gene itself. That is to say, there may be two or more different main genes, each giving a characteristic mean age of onset, with some variation round it.

(2) Differences in modifying genes, which, while not causing the disease, may favour early or late onset if a main gene is present.

(3) Differences in environment, which again may favour early or late onset.

The recent work of Bell (1934, 1935, 1939) enables us, for the first time, to answer this fundamental question in certain cases. Bell takes a large group of patients, and finds the correlation between age of onset in pairs of sibs (and sometimes also in parents and offspring). Let us see what we should expect on various extreme hypotheses. It is not

suggested that any one is ever exactly true, but one or other may prove a good approximation to the truth in a particular case.

If the age of onset were determined wholly by the main gene, and if there were only one main gene, we should expect to find the age of onset exactly the same in all cases, say 7 years. But if there were several different genes we should expect to find several different ages of onset, say one group at 4 years, another at 12, and another at 30. If there were several different recessive allelomorphs, some ages of onset would characterize homozygotes, and these would almost invariably be found where the parents were related. Where they were unrelated we should sometimes find heterozygous "compound" recessive types, probably with intermediate ages of onset. In every case of dominance parent and offspring would be affected by the same gene, and have the same age of onset. Thus the correlation would be unity. Two sibs would always have the same pair of recessive main genes except where one parent was affected, and happened to be a heterozygous compound. Thus the correlation between sibs would be very close to unity.

Next suppose that only one main gene were present in the population, but that there were also a number of modifying genes which affected the age of onset. For example, glaucoma is due to abnormally high pressure within the eye. We might expect the gene or genes which are responsible for high arterial pressure to accelerate its onset. If there were only one main gene and a considerable number of modifiers we should expect to find the same situation as with characters such as stature, which appears to be controlled by many genes. The correlation coefficients would be about 0·5, probably a little higher for sibs, and a little lower for parents and offspring. In the case of a dominant gene, the normal parent would, on the average, be responsible for just as much modification as the affected parent, though we should in general have no clue as to the nature of the modifiers to be found in a normal individual.

If there were one main gene and no modifiers, but environment played a large part in determining the age of onset, we should expect to find some correlation, but its value would be low and uncertain. It would be much higher between sibs than between parent and offspring. For sibs are generally brought up in a similar environment, but the family environment may change a great deal within a generation. It would also be higher in a disease manifesting itself in childhood than later in life, since sibs are brought up in the same home and may separate later on. If environment were important in determining the age of onset we might

expect to find this fact recognized, and there might be large occupational and sex differences, as there are with cancer, which undoubtedly has a genetical basis in many instances. The influence of the environment is recognized in the case of the congenital photosensitivities, such as haematoporphyria and xeroderma pigmentosum. If there were no genetical basis for correlation, we should expect to find coefficients well below 0·5 in most cases.

Bell's findings are summarized in Table 1. In no case except the first is there any marked sex difference in frequency or in age of onset.

Table 1. *Correlation coefficients of ages of onset (after Bell)*

Disease	Type	Pairs of siblings		Parent and offspring	
		n	r	n	r
Optic atrophy (males only)	S.L. Rec.	812	0·510	—	—
Glaucoma	Dom.	256	0·897	113	0·813
Huntington's chorea	Dom.	442	0·465	153	0·593
Peroneal atrophy	Dom.	164	0·803	0·8	0·764
	Rec.*	108	0·840	—	—
Friedreich's ataxia	Dom.	144	0·925	—	—
	Rec.	500	0·694	—	—
Spastic ataxia	Dom.	198	0·812	—	—
	Rec.	164	0·845	—	—
Spastic paraplegia	Dom.	154	0·884	—	—
	Rec.	218	0·852	—	—
Grouped ataxias and paraplegia	Dom.	—	—	135	0·743

* Includes a few sex-linked cases.

The last line refers to grouped cases of dominant Friedreich's ataxia, spastic ataxia, and spastic paraplegia, which in Bell's opinion, though not in my own, may be manifestations of the same fundamental inherited abnormality. The precise value to be attached to the significance of these coefficients is rather uncertain, because except in the case of Huntington's chorea, the distribution of ages of onset is highly asymmetrical, and the theory of normal correlation does not apply. However, there is little doubt that in most cases the values are significantly above 0·5. Thus in the case of recessive Friedreich's ataxia, the value of Fisher's (1938) transformed coefficient z is $0·856 \pm 0·045$. This differs from $z = 0·549$, the value corresponding with $r = 0·5$, by nearly seven times its standard error. In the case of dominant peroneal atrophy, when parents and offspring are compared, $z = 0·996 \pm 0·109$, and the difference from 0·549 is over four times its standard error. On the other hand, the difference between the transformed fraternal and parental correlations for Huntington's chorea is about 1·9 times its standard error, and therefore not quite significant.

Actually these coefficients are, if anything, underestimated. Bell points out that in the case of the ataxias and spastic paraplegia the age of onset is, on an average, earlier in younger than elder sibs, perhaps because the parents are on the look-out for signs of the disease. Thus the coefficient of correlation is slightly raised if the table is made asymmetrical, age of onset being correlated in elder against younger sibs.

She has kindly calculated the effect of this in the case of recessive Friedreich's ataxia, and finds that it is raised from 0·694 to 0·711. Further, she has separated the dominant and recessive forms of the same disease which cannot be distinguished clinically. The ages of onset in the two groups overlap, but it is much later on the average in the dominants. If this had not been done, the correlations would be much higher. Thus the coefficient for dominant and recessive spastic ataxia combined is 0·890.

The calculation of the correlations between age of onset in sibs may be regarded as an analysis of variance, the variance within sibships being small compared with that between sibships when r is high. Thus in the case of dominant Friedreich's ataxia, $0·925^2$ or 85·6 % of the variance is between sibships, only 14·4 % being within them.

I think that there is no escape from the conclusion that several different main genes are concerned in the causation of the diseases other than optic atrophy and Huntington's chorea. On no other hypothesis could the correlation coefficients be so large. The argument is particularly clear in the case of parent-offspring correlation. When the age of onset of glaucoma in a parent is known we have eliminated a fraction $1 - r^2$, or 0·661 of the variance in the ages of onset in the offspring. Suppose that both parents influence this age by contributing modifiers, this fraction would be expected to be about $\frac{1}{4}$, since half the modifiers from each parent are lost by segregation. Even if we neglect segregation, there is not enough variance left over for the normal parent's modifiers to produce unless we postulate assortative mating so intense as to give rise to a correlation coefficient of over 0·5 for the modifying genes in the parents.

If we could distinguish the different main genes we could reduce the correlation coefficients to more normal values. Thus by dividing up the genes responsible for spastic ataxia into a dominant and recessive class, Bell was able to reduce a coefficient of 0·890 to two of 0·845 and 0·812. If we divide the pedigrees of dominant spastic ataxia into two groups, in one of which the age of onset is always under 50, in the other always over, we obtain two correlation tables, with $n = 162$, $r = 0·562$, and $n = 36$,

$r = 0.027$. It is not of course suggested that this represents a real genetical division, but it happens that in this particular table all sibships can be divided into those with ages of onset over or under 50.

In the same way we can divide her correlation table for dominant Friedreich's ataxia into three parts. She considered seventy-two pairs of sibs, so there were 144 entries in the table. In the forty cases where the age of onset in one sib was under 5, it was invariably so in the other. In the twelve cases where it was between 35 and 39 in one sib, it was also so in the other.

The argument for the existence of numerous genes is made all the more plausible because Bell has already shown that in the last four diseases of Table 1 there is a dominant and a recessive form. Besides these there is a sex-linked recessive form of peroneal atrophy, and in one pedigree (490) spastic paraplegia behaves as a sex-linked recessive. If then three different genes may be responsible for a group of cases which are indistinguishable clinically, why not five or six? The different genes may in some cases be allelomorphic. The demonstration of this must await linkage tests. If the argument of Haldane (1940a) is accepted, recessive spastic paraplegia is due to a group of partially sex-linked genes, and their allelomorphism is highly probable. The dominant form is due to autosomal genes.

Pedigree 464 (due to Fry) suggests an interesting possibility. It is shown in Fig. 1. The grandfather of the three affected children was healthy up to 65, when his gait became uncertain. At 86 he had marked
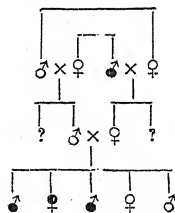
Fig. 1.

ataxia and other symptoms. He lived till 91. The grandchildren developed the disease at the age of about 10. As their parents were double first cousins we naturally suspect a recessive gene. It seems likely that the affected grandfather and his sister or her husband were both heterozygous for it, as were the parents of the affected sibs, but that it only showed in one heterozygote, and that very late in life. Examples can be quoted, particularly from *Drosophila*, where a gene generally behaves as a recessive, but occasionally shows up as a "weak" dominant.

Similarly, in case 499, a woman who had married her uncle had a spastic paretic gait at the age of 72, but except for one attack in middle life, could walk without sticks till 62. Three daughters developed symptoms at about 40, and were severely crippled at 50. Several other cases, for example 385, where there is no inbreeding, are nevertheless suggestive of the same possibility, namely, that some of the genes concerned are incompletely recessive.

No doubt, even in the cases of high correlation, modifying genes may play a part. Bell's correlation tables show a few striking outliers. Thus in the case of recessive Friedreich's ataxia there were three cases out of 250 where the age of onset in sibs differed by over 25 years. If these were omitted from the table, the coefficient of correlation would be raised from 0·694 to 0·802. There is a strong suggestion that about 1% of the population may carry modifying genes which markedly delay the onset of this disease if the main gene is present.

Dr Bell has kindly permitted me to use a table prepared by her of the differences in age of onset of the disease in pairs of sibs. Besides calculating the mean difference we may proceed as follows. If each family were large enough there would be a mean age of onset in affected members, and a distribution round this mean. We can readily determine the even cumulants of this distribution.

These cumulants are half those of the differences, if we count each difference both as positive and negative, and make Sheppard's correction. Thus if ν_2 and ν_4 are the mean values of the squares and fourth powers of the differences, we have, for the distribution about the family mean,

$$\sigma^2 = \tfrac{1}{2}\nu_2 - \tfrac{1}{12}, \quad \gamma_2 = \beta_2 - 3 = \frac{2\nu_4 - 6\nu_2^2 + \tfrac{1}{30}}{(\nu_2 - \tfrac{1}{6})^2} = \frac{2(\nu_4 - \nu_2 + \tfrac{1}{10})}{(\nu_2 - \tfrac{1}{6})^2} - 6.$$

The unit of grouping is taken as a year. Actually it is larger in a fraction of the cases. Thus the true value of σ is slightly smaller than that given, the true value of γ_2 slightly larger. However, as Sheppard's correction never amounts to 2%, the further correction to be made is small. The results of this calculation are shown in Table 2.

Table 2. *Differences of age of onset within sibships*

Disease	Type	Mean	S.D.	γ_2
Friedreich's ataxia	Dom.	2·61	3·10	+ 6·52
	Rec.	2·47	3·38	+39·6
Spastic ataxia	Dom.	6·03	6·04	+ 2·47
	Rec.	3·51	6·14	+21·2
Spastic paraplegia	Dom.	3·99	5·01	+12·5
	Rec.	3·01	5·91	+26·5

A somewhat more accurate value of γ_2 could be obtained from the complete data. The standard deviation round the family mean varies between 3 and 6 years, and the values of γ_2 are all positive, and are large in the case of recessives. This means that there are fewer small and more large deviations than in a normal distribution with the same standard deviation, particularly in the case of recessives. This is what we should expect if there were a few modifying genes with considerable effect, but in most families there was very little modification. It is, however, curious that modifiers for recessives seem to be more frequent or more effective than those for dominants.

There is some evidence for the existence of modifying genes in the case of Huntington's chorea. Bell found that of 385 affected males 60·3% had affected fathers, and of 336 affected females 51·5% had affected mothers. If we consider grandparents also we have the results of Table 3.

Table 3. *Huntington's chorea*

	Affected		Normal		
Affected ancestors	Males	Females	Males	Females	q
Mother and grandmother	36	47	29	24	0·559
Mother and grandfather	43	59	32	32	0·548
Father and grandmother	36	36	24	41	0·438
Father and grandfather	92	55	53	77	0·394

In this table normals are not included unless they appear to have reached the age of 30. q is the frequency of affected females plus normal males. These results are consistent with the theory (Haldane, 1936) that some of the genes which modify the age of onset are sex-limited in their effect. Thus if there are modifiers in a family increasing the age of onset in females there will be an excess of males among the affected and of females among the normal. This will account for the results tabulated. The differences between q values are not all significant, but their order is as expected.

DISCUSSION

This paper is of course of a preliminary nature. Data for ages of onset must exist for a number of diseases. Ages of death would in some cases be equally valuable or more so, since here there is no subjective element. Any other measurable character of the disease would be equally valuable, provided it is fairly definite. Thus in pedigrees of myopia we could use the strength in dioptres at some standard age, in dwarfism the

height, and so on. But a very variable character, such as the coagulation time in haemophilia, would be useless.

Differences in age of onset in man probably correspond with differences in penetrance in *Drosophila* or other insects. If we imagine all cases in a pedigree to be examined at the age of 25 only, late age of onset would appear as low penetrance. On Goldschmidt's theory genes act by determining the rates of processes. In man a morbid process may lead to manifest disease at any age. In an insect an abnormal developmental process will not produce any visible effect on the morphology unless it does so before the imago is fully formed. Thus a study of ages of onset can tell us a good deal more than a study of penetrance for equal numbers studied.

So far as concerns the question of evolution, Huntington's chorea seems to agree with the conditions postulated by Fisher (1931) in his theory of the evolution of dominance. Modifiers are presumably being selected which delay the age of onset. Perhaps Huntington's chorea was a disease of infancy in *Sinanthropus*. And if eugenic measures are not taken against it, it may be confined to old age in our remote descendants, in which case the main gene will spread, and homozygotes appear, so that it will be, in effect, a recessive disease. But in the case of the other conditions (except Leber's disease, which is either sex-linked or cytoplasmic) the main effect of selection will be to weed out those main genes which produce an early onset, particularly where the disease is dominant.

This is discussed in detail elsewhere (Haldane, 1940*b*). As pointed out by Bell, selection causes the dominant forms to be less severe than the recessives. Modifying genes may exist, but where the average difference in age of onset between sibs is only 2 or 3 years, they cannot be very important, and must be selected very slowly. A great deal more work will be required before we can judge whether the presence of modifiers in accordance with Fisher's theory is common or rare in the human species.

Summary

Bell's data on the age of onset of some human hereditary diseases are discussed. In glaucoma, peroneal atrophy, Friedreich's ataxia, spastic ataxia, and spastic paraplegia, the age of onset in all affected members of a pedigree is nearly the same, while different pedigress differ widely. Thus a number of different main genes must be responsible for the clinically indistinguishable diseases in different families. In optic

atrophy and Huntington's chorea the differences of age of onset within a pedigree are nearly as large as those between different pedigrees. So the same main gene may be responsible for all cases, while modifying genes account for much of the difference in age of onset. The bearing of these facts on evolutionary theories is discussed.

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VARIATION AND SELECTION OF POLYGENIC CHARACTERS

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(With Plate 6 and Ten Text-figures)

CONTENTS

1. INTRODUCTION

GENETICS has been mainly concerned with the inheritance and behaviour of genes by means of which individuals can be classified into categories showing sharp phenotypical differences. These are the so-called "qualitative" genes. It has, however, long been recognized that there exists another type of heritable variation, termed "quantitative" or "metrical".

11-2

Such variation does not allow of individuals being separated into distinct types; all gradations between certain limits are to be observed. The only classifications possible are arbitrary and dependent on the accuracy of the available means of measurement. Stature in man is typical of this kind of character. Human stature is, indeed, of special interest, as it is one of the characters whose study led to the formulation of Galton's law of ancestral inheritance and to the dispute on the nature of inheritance between Pearson and Bateson.

Early researches on these quantitative or metrical characters were devoted mainly to discovering the mechanism of their inheritance, whether blending, as the biometricians supposed, or particulate, as in the case of the sharply distinguished variants. It is now generally accepted that these characters are (*a*) controlled in inheritance by an indefinitely large number of genes, many of which have approximately equal effects, and (*b*) markedly subject to environmental variation. Thus it is clear that characters of this kind will be difficult to study by the common techniques of genetics. Some method of distinguishing between environmental and genetical variation must be used, and the analysis of the genetical variation itself will be complicated by the impossibility of separating the large number of genes involved. Complex statistical methods are needed. For these reasons the genetical analysis of such characters has progressed very slowly, in spite of their vital importance for the theory of evolution and the practice of selection.

The distinction between qualitative and quantitative variants is itself not a final one. It is possible that, if some organism could be grown in a constant environment and rendered homozygous for all but one of the genes affecting a quantitative character, this one gene might be observed to segregate and give sharply distinct classes just as a qualitative gene does. Nor do qualitative and quantitative genes affect different characters. Stature, for example, is usually a quantitative character, but in many organisms dwarf forms are known to segregate sharply from the normal type, so falling into the qualitative class. In any case most variations are in some sense quantitative, and so the terms qualitative and quantitative are far from ideal. A better classification can be made on the basis of the mode of inheritance. Qualitative variation is usually monogenic or digenic in inheritance. Cases of trigenic and tetragenic inheritance are known, but are relatively rare. In contrast with these, quantitative variation may be said to be polygenic, and this term will be adopted.

The importance of a better knowledge of polygenic inheritance can

hardly be overestimated. Geneticists have found it convenient to use monogenic characters in the study of chromosome behaviour, of mutation, and of gene action; but to those who are concerned with applied genetics, in plant and animal improvement as well as in the study of evolutionary changes, such characters are of minor importance, most of the variation being polygenic. It is true that wild populations of a number of organisms are known to be heterogeneous for monogenic characters, but these are usually rare variants. It is, on the other hand, highly probable that all populations are heterogeneous for polygenic characters. Furthermore, specific differences are always polygenic if the species are biologically isolated. Some cases of monogenic specific differences have been described, notably in wheat (Watkins, 1930), but in most cases, as for example *Antirrhinum* (Baur, 1924) and *Nicotiana* (East, 1935), polygenic differences have been observed. This would also appear to apply to the distinguishing features of the two races of *Drosophila pseudoobscura*, especially to the mechanism determining inter-racial sterility (Dobzhansky, 1936). This view derives further support from the observation of homologous monogenic variants, both naturally occurring and mutants from culture, in the *Drosophila* species (Gottschewski & Tan, 1938; Sturtevant & Tan, 1937). If such monogenic changes were the materials for specific differentiation, as their occurrence in the wild has suggested to some authors, at least some of the species should differ by these genes. Their occurrence as homologous mutations in several of the forms shows that the latter do not, however, differ in this way. Thus a study of polygenic rather than monogenic inheritance is the prime need of applied genetics.

There are a number of ways in which the problem can be approached, but perhaps the most appropriate is by an analysis of the effects of selection. Clearly the kind of result of selective breeding is a compound of the nature of the selection applied and the nature of the genetic variation available for the action of selection. The type of selection can be controlled by the experimenter, within reasonable limits, and so definite inferences about the nature of the available variation can be drawn from the results of such a programme. A series of such experiments is described and discussed below.

2. Preliminary investigations

Drosophila melanogaster was chosen as the material for this investigation, for two reasons. In the first place it has the technical advantages of being easy of culture and a rapid breeder. Secondly, the

existence of tester stocks makes possible the further genetic analysis of any selected lines, should such analysis be desirable.

Of the various characters which are most probably polygenic in inheritance the one eventually chosen was the combined number of chaetae on the ventral surfaces of the fourth and fifth abdominal segments.[1] The numbers of hairs on these segments are easily determined under a high-power binocular microscope, as will be seen from the photographs of Pl. 6, and as there are usually from thirty-five to forty-five hairs on the segments taken together, the discontinuity of the distribution leads to no serious difficulty. The joint number of hairs of the two segments was used as the metric throughout all the experiments. In the original stocks there was no evidence of differences between the means of the segments taken separately, but this point was not tested in the selected material. Hence it is not possible to say whether the segments made equal contributions to the joint effect of selection.

The number of hairs per fly varies continuously from about thirty to about fifty in the original stocks, in the sense that flies with all numbers of hairs between these limits can be found. (These limits were later widely transgressed in selected material.) Some examples of the frequency distribution of hair number are shown in Text-fig. 1. It will be
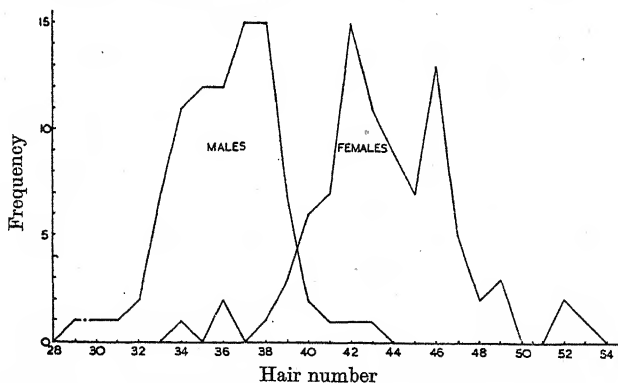
Text-fig. 1. Frequency distributions for hair number in males and females of the **BB** stock.

seen that the curves approximate to normality, being in fact typical of the frequency distributions observed for polygenic characters. In particular there is no consistent evidence of skewness, as tested by the k_3 statistic (Fisher *et al.* 1932). Nor is bimodality regularly encountered.

[1] According to Bridges' illustrations, the fifth is the most posterior hair-bearing segment in the male; but in the female the sixth also carries some hair.

Hence the distributions are reasonably well described by calculations of their means and variances. These are tabulated for a number of the original stocks in Table 1. It will be seen that the mean number of hairs is consistently lower in males than in sister females. Furthermore, the stocks show different means, thus providing *prima facie* evidence of hair number being a polygenic character. It is, however, also clear that some non-heritable variation occurs, since the Oregon+ line, inbred for at least seventy generations, still showed considerable variation.

Two questions now arise, viz. as to whether heritable variation occurs and, if so, whether it is polygenic. That the variation within other stocks was not wholly non-heritable was shown by a series of single generation selection experiments. Pairs of flies with high, medium and low hair number were chosen from certain stocks and mated. It was observed that, in all but the Oregon+ line, there was a positive regression of the progeny mean on the parental values, thus showing that the variation in hair number is partly heritable. It was also observed that the progeny means did not fall into clearly delimited groups, so showing that the character is polygenic. The progeny means showed no relation to the parental values in the Oregon+ line, thus indicating that the inbreeding this line had undergone had in fact made it homozygous for the hair-number polygenes. Thus the character is quite suitable for a study of the action of selection in polygenic inheritance.

It may be added that the hair number also constitutes a specific difference. Table 1 shows the mean numbers of various species other than *D. melanogaster*. These means show marked differences. The sex

Table 1

Stock	Males			Females		
	No. counted	Mean	Variance	No. counted	Mean	Variance
f B^{i}B^i	20	31·85	7·5026	20	36·95	10·8921
B	20	36·85	8·0289	20	42·25	8·6184
BB	20	35·45	6·0500	20	42·60	5·9368
Oregon+	20	39·55	4·1553	20	44·40	4·8842
y × w m f	20	37·90	5·8842	20	42·40	12·9895
D. simulans	10	31·50	5·1667	13	40·7692	9·1923
D. subobscura	20	30·75	7·8816	20	33·25	5·7763
D. virilis virilis*	20	59·90	12·7263	20	34·90	3·9895

All figures refer to the sum of the hair numbers of the fourth and fifth segments.

* In *D. virilis virilis* only one segment (no. 5) was counted. Hence the means in the table should be multiplied by two to render them comparable.

difference in hair number is also variable between species. In *D. virilis* it is the reverse of that found in *D. melanogaster*. So species differentiation

in *Drosophila* has involved the production of hair number differences, presumably by the action of selection, as such a difference as that between *D. virilis* and *D. subobscura* cannot reasonably be ascribed to chance fluctuations. Thus selection experiments on hair number are in some sense a repetition of the action of natural selection in species formation.

3. The cross $\underline{y} \times f\,B^iB^i$

(a) *Technical*

Two crosses were used in the main experiments, viz. $\underline{y}$ females from the $\underline{y} \times w\,m\,f$ stock by $f\,B^iB^i$ males, and Oregon+, inbred, reciprocally crossed with BB. Though actually the second in time, the former cross will be described first as it proved less complex than the latter.

The initial cross was of $\underline{y}$ females by $f\,B^iB^i$ from stock. The mating was made in a vial. The flies were transferred to a half-pint milk bottle after a day, and were allowed to lay in this bottle for several days. Their progeny were counted and two F_2 cultures set up, each comprising a mating of two females by two males taken at random from the F_1. These F_2 matings were made in vials and after 1 day transferred to bottles, where they were allowed to lay for 2 days. This procedure was followed in all the subsequent matings, with the exception that single pair matings were used to give the F_3 and later generations.

The hair number of the F_2 flies was counted and the two highest females mated individually to the two highest males to give a high selected F_3. Similarly, the two lowest females were mated individually to the two lowest males to start the low selection line. These two lines, the high and low selections, were maintained separately in later generations by selecting the two highest, or lowest, females and the two highest, or lowest, males in each generation in order to make the two single-pair matings from which the next generation was bred. Thus the two progenies in the fourth generation of the high line were from the two highest males and females of the third generation of that same line, and so on. As far as possible each mating was made between a female and a male from different bottles of the previous generation, in order to avoid inbreeding. In the later stages of the experiment a considerable degree of sterility was encountered. The progenies were small and many matings failed completely. To mitigate this difficulty, three matings per generation were made in each line, though more than two progenies were seldom counted or used to provide parents of the next generation. Where all three matings were fertile, the two with the most extreme parents, viz. the highest in the high line, and the lowest in the low line, were counted

and selected for the next generation parents. Finally, the high and low lines were kept in step throughout the experiment, i.e. the corresponding generations of each line were always raised side by side in the incubator, which was maintained at a temperature of $25 \pm 1^\circ$ C. Never more than twenty flies of each sex were counted from a single culture.

The means of the cultures in the various generations of each line, together with those of the parental stocks, are given in Table 2. The males and females are given separately. Text-fig. 2 shows the means

Table 2. *The cross* $\underline{y} \times \mathbf{f\ B^i B^i}$

Generation	Males No. counted	Males Mean	Females No. counted	Females Mean	Generation	Males No. counted	Males Mean	Females No. counted	Females Mean
Parent f $B^i B^i$	20	31·85	20	36·95	H 8—1	14	38·08	12	46·50
Parent $\underline{y} \times \mathbf{w\,m\,f}$	20	37·90	20	42·40	L 1—1	9	34·67	9	42·56
F_1	14	34·36	18	38·72	—2	5	34·80	7	40·00
F_2—1	20	35·00	20	41·10	L 2—1	20	34·95	20	41·60
—2	20	35·40	20	42·95	L 3—1	20	36·05	18	44·33
H 1—1	9	35·67	13	44·31	—2	20	34·70	20	39·95
—2	9	38·56	11	47·45	—3	20	34·00	20	40·35
H 2—1	12	38·83	9	46·22	L 4—1	13	34·23	10	39·90
—2	13	37·69	10	46·30	—2	20	34·35	20	39·55
H 3—1	10	40·50	6	45·40	—3	20	35·00	19	40·42
—2	15	38·47	11	44·81	L 5—1	20	33·90	20	39·35
H 4—1	5	37·20	11	46·73	—2	13	33·62	15	38·67
—2	13	38·39	7	45·43	L 6—1	8	34·13	8	40·88
H 5—1	13	38·54	20	45·05	—2	9	34·32	20	40·35
—2	10	38·50	9	45·33	L 7—1	9	34·56	13	39·92
H 6—1	13	39·15	14	45·00	L 8—1	10	34·30	14	40·29
H 7—1	13	38·08	12	46·50	—2	13	32·23	15	40·47

H = high line. L = low line. The number of the generation is the number of occasions in its ancestry at which selection has been exercised.

The various cultures in each generation are shown separately.

plotted against generation. Each dot represents a culture mean, except in a few cases where the two cultures each gave so few flies that they had to be added together to give a reasonable number of individuals. The continuous lines in Text-fig. 2 join the means of the means. These were not obtained by weighting according to the number of individuals involved, i.e. they are *not* the grand means of each generation in each line. They were calculated by taking the unweighted culture means, adding, and dividing by the number of cultures involved, irrespective of how many flies each culture had yielded.

(b) Results

The F_1 flies were intermediate in hair number between the parental lines, but the F_2's showed an increase over the F_1's. This is most probably

due to the fact that the F_1 culture was overcrowded, as the parents were allowed to lay in the bottle for several days, whereas egg-laying in the F_2 and later generations was limited to 2 days. The hair numbers of the
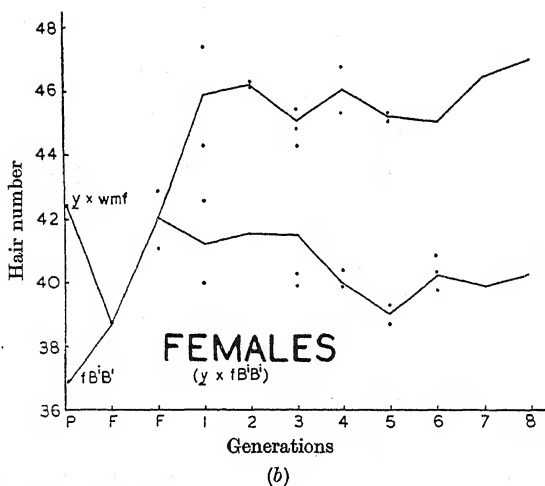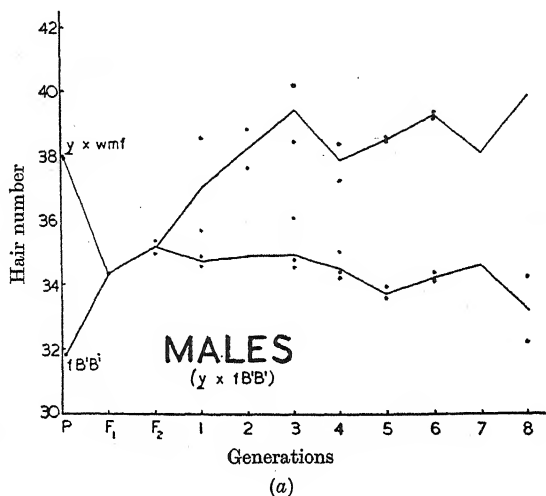
Text-fig. 2. The effect of selection for high and low hair number in $\mathbf{y} \times \mathbf{f\,B^i B^i}$. Each dot is a culture mean and the lines join means of means. (See also Text-fig. 3.)

parental stocks are comparable with that of the F_1, as they were some-what crowded too.

The selection for hair number had an immediate effect, the high and low F_3's differing markedly. The second selected generation (F_4) showed an even larger difference between high and low, but after that point

little change is apparent in Text-fig. 2. It will be seen, however, that there is considerable fluctuation in each line from generation to generation, especially in the females. This is almost certainly due to environmental causes and can be largely eliminated by plotting the difference between the high and low lines instead of the actual means. This is done in
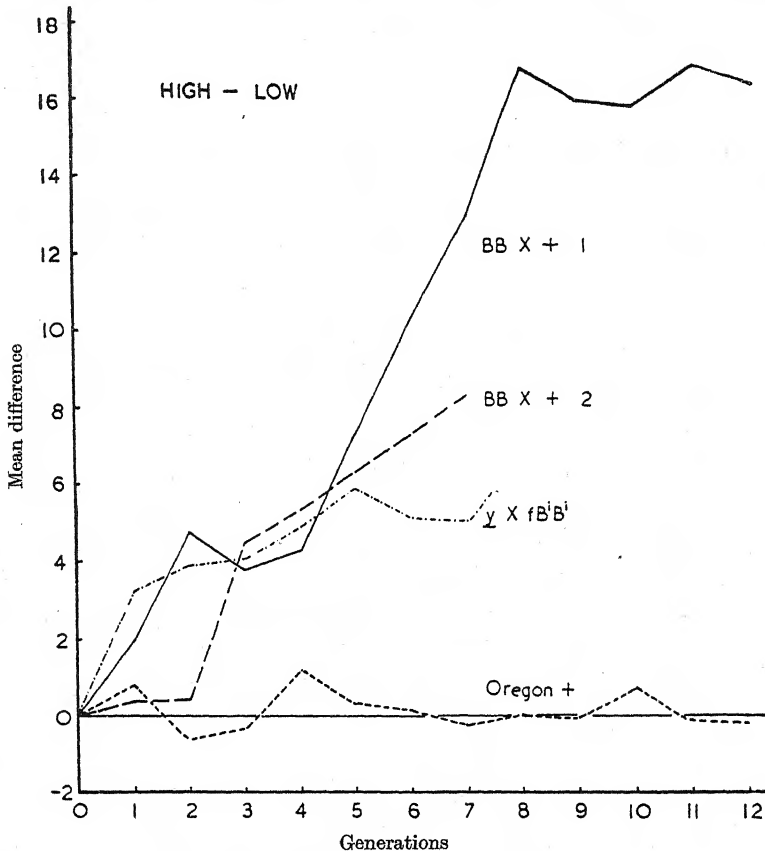
Text-fig. 3. The mean difference between high and low selection lines in various stocks plotted against generations of selection. (Generation $0 = F_2$ in the case of crosses.)

Text-fig. 3. The average difference for each generation was obtained by subtracting the mean of the low females of that generation from the corresponding female mean in the high line. The males were treated similarly and the two differences averaged. This smooths out most of the environmental fluctuation and makes interpretation of the results materially easier. The first generation plotted in Text-fig. 3 is the F_2 from which the high and low lines originated. The average difference in

F_2 clearly must be zero. The difference clearly increases up to the second selected generation and afterwards is fairly stable, though perhaps showing a slight tendency to increase. The effect of selection was however almost complete after two generations, and was presumably confined to choosing new combinations of whole chromosomes.

Two points must be added, firstly that the main effect of selection was to increase the high line hair number, rather than to decrease that of the low line and secondly that the final difference between the high and the low lines was no greater than that shown by the parental stocks.

4. The cross $\mathbf{BB} \times +$

(a) Technical

The technique used for this cross and the breeding lines selected from it was precisely the same as for $\mathbf{y} \times \mathbf{f}\ \mathbf{B^l B^l}$ with the exception that in the initial matings, as well as in all the subsequent ones, single pairs were allowed to remain only 2 days in the culture bottle. Thus only the hair numbers of the parental stocks, $\mathbf{BB}$ and inbred Oregon$+$, were obtained from relatively crowded cultures.

The two stocks used differed slightly in their mean hair numbers, $\mathbf{BB}$ being somewhat lower in both sexes than the inbred Oregon$+$ stock. The reciprocal F_1's were both intermediate on the whole between the parental stocks (Table 3 and Text-fig. 4). The means of the twelve F_2 cultures, six from each of the reciprocal F_1's, resembled those of the F_1: One point of special interest was observed in the F_2 cultures. The males were showing segregation for $\mathbf{BB}$ and $+$, and the former kind showed a higher hair mean number than the latter (Table 4). A suitable test of significance was applied to the results. If there is no real difference between the hair numbers of $\mathbf{BB}$ and $+$ males, the mean difference between the values of the two classes in individual cultures will be zero. Twelve such differences are obtainable from the twelve F_2 families. The mean of the twelve values ($\bar{d}$) is 1·1350. The variance of this mean ($V_{\bar{d}}$) is 0·1182, and $t = \bar{d}/\sqrt{V_{\bar{d}}} = 3\cdot301$ for eleven degrees of freedom. This has a probability of less than 0·01 and so it must be judged that $\mathbf{BB}$ males have a higher hair number than their $+$ brothers.

In six of the F_2 cultures, viz. those from the cross $\mathbf{BB}/+ \times +$, the $\mathbf{BB}$ segregation was also followed in the female progeny. In this case too the $\mathbf{BB}/+$ females had a higher mean hair number than their $+$ sisters, as $\bar{d} = 0\cdot8238$, $V_{\bar{d}} = 0\cdot08619$ and $t = 2\cdot806$ for five degrees of freedom, giving a probability of between 0·05 and 0·02; the difference

between differences of the two sexes is not significant. Thus although the **BB** parental stock had a lower hair number than the Oregon+ to which

Table 3. *The cross* **BB** × +

Generation	Males No. counted	Males Mean	Females No. counted	Females Mean	Generation	Males No. counted	Males Mean	Females No. counted	Females Mean
Parent **BB**—1	20	35·45	20	42·60	H 8—1	20	46·20	20	51·65
—2	20	37·60	20	46·45	—2	20	42·85	20	48·60
—3	19	35·05	16	41·75	H 9—1	20	44·65	20	49·60
—4	7	36·00	16	42·75	—2	13	44·38	20	48·90
—5	20	36·20	13	43·85	—3	20	43·75	20	49·25
Or+—1	20	40·65	20	45·20	H 10—1	20	42·70	20	49·35
—2	20	39·55	20	44·40	—2	20	44·15	20	47·30
—3	20	38·45	20	43·75	—3	20	44·55	20	48·70
—4	20	40·90	20	45·05	H 11—1	20	43·65	20	47·65
—5	20	39·85	20	44·55	—2	7	44·86	15	50·20
F_1: + × **BB** —1	20	37·75	20	42·30	H 12—1	20	44·80	20	49·00
—2	20	37·50	20	44·70	—2	9	41·89	10	49·30
—3	20	38·60	20	42·95	H 13—1	20	44·85	20	51·40
BB × + —1	20	38·05	20	43·00	L —1	20	37·60	20	44·00
—2	20	37·25	20	44·05	—2	15	37·67	15	44·13
F_2: ex+ × **BB** —1	23	39·35	6	45·43	—3	20	37·05	20	42·90
—2	40	38·65	29	44·76	L 2—1	20	36·55	20	41·60
—3	38	38·87	40	44·30	—2	20	36·75	20	40·60
—4	21	39·00	13	45·15	—3	20	35·55	20	40·82
—5	35	38·77	21	43·90	L 3—1	11	36·30	9	41·44
—6	32	38·78	26	44·88	—2	20	35·75	18	41·24
ex**BB** × + —1	30	36·87	21	44·05	—3	20	35·00	20	43·20
—2	40	38·78	36	43·50	L 4—1	20	36·85	20	42·15
—3	40	38·45	31	43·74	—2	16	35·25	20	40·60
—4	40	36·63	17	43·24	—3	16	34·25	20	40·50
—5	22	39·14	16	44·06	L 5—1	13	35·31	20	40·50
—6	40	36·90	30	43·47	—2	20	34·90	20	40·30
H 1—1	16	39·31	20	44·95	—3	20	32·20	20	38·05
—2	20	40·80	20	44·40	L 6—1	20	32·85	15	38·53
—3	20	39·40	20	46·40	—2	20	32·65	15	38·93
H 2—1	20	40·05	20	46·10	L 7—1	14	30·64	14	34·93
—2	20	40·00	20	46·40	—2	15	30·33	20	36·05
—3	18	40·33	20	44·25	L 8—1	20	26·30	20	31·60
H 3—1	20	40·15	20	46·45	—2	20	28·65	20	34·50
—2	20	39·65	20	45·00	—3	20	28·40	20	34·25
—3	20	38·40	20	45·95	L 9—1		Very few flies		
H 4—1	16	38·94	10	44·80	L 10—1	12	27·67	23	32·70
—2	20	39·70	20	46·45	L 11—1	3	23·67	19	30·89
H 5—1	20	39·60	20	45·65	—2	16	27·31	20	33·00
—2	20	41·25	20	50·20	—3	20	26·55	20	31·35
H 6—1	14	43·21	13	48·85	L 12—1	7	25·71	12	32·92
—2	7	42·29	7	48·86					
—3	13	42·69	9	48·78					
H 7—1	26	43·47	38	49·58					
—2	20	42·80	20	48·00					

Nomenclature as in Table 2.

it was mated, the *X* chromosome of **BB** had genes potentially able to give a higher hair number than that on the Oregon *X*-chromosome. The autosomes of **BB** must then have had sufficient low hair number genes

MALES
(BB × +)

(a)

FEMALES
(BB × +)
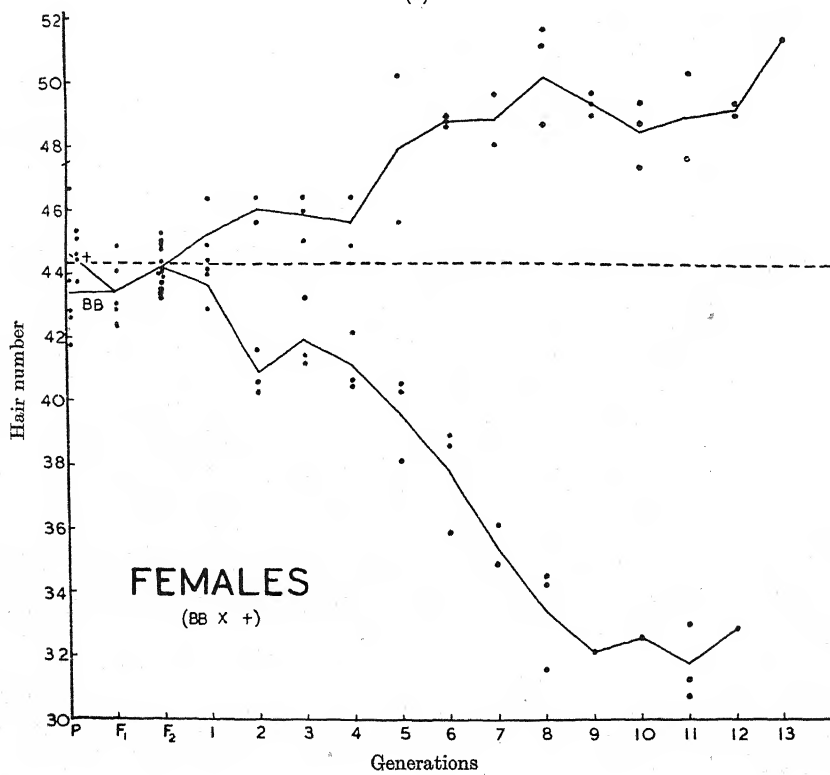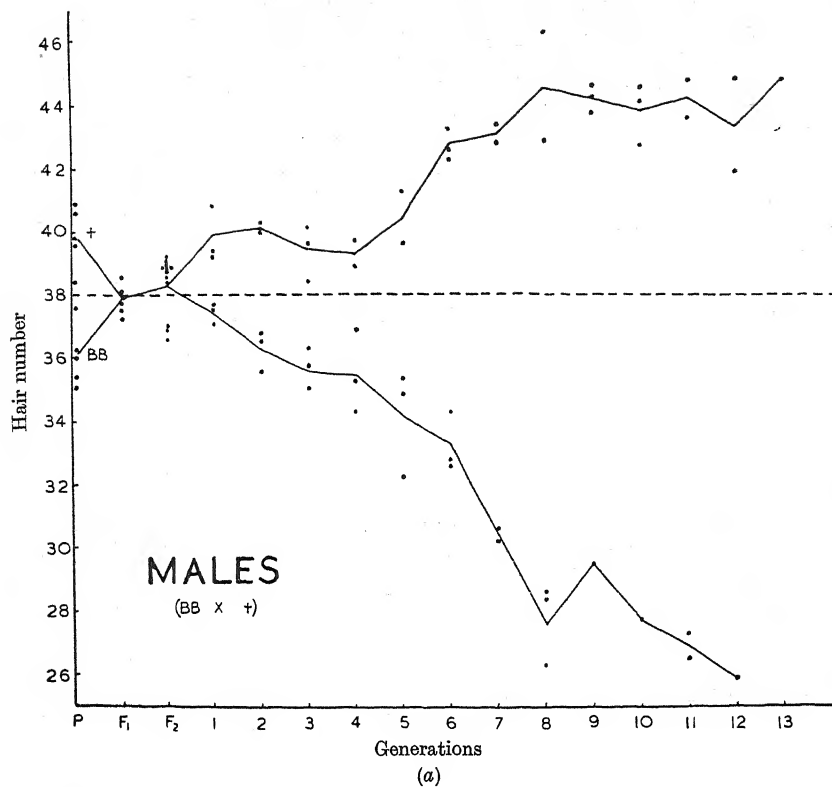
to outweigh the effect of the X. The existence of such a difference between the gene contents of **BB** and **+** sex chromosomes was confirmed by the fact that, though eye shape was neglected in selection, the high line was soon homozygous **BB** and the low line **+**.

In order to raise the F_3 generation a large number of matings were made between F_2 males and females chosen at random. The mean hair numbers of the various F_3 progenies was determined and can be used to calculate the regression of hair number in offspring on that in parents. These calculations are, however, outside the scope of the present account

Table 4. *Hair numbers of* **BB** *and* **+** *flies in* F_2 *of* **BB** $\times$ Or **+** *and reciprocal*

	Culture	Mean of BB	+	(d) difference	
Males:	$F_2\,ex$**+** $\times$ **BB**—1	41·3300	37·3636	3·9664	$\bar{d} = 1·1350$
	—2	39·5500	37·7500	1·8000	$V_d = 1·4187$
	—3	39·2000	38·5000	0·7000	$V_{\bar{d}} = 0·11822$
	—4	39·0000	39·0000	0·0000	$s_{\bar{d}} = 0·3438$
	—5	39·8000	38·0000	1·8000	$t = 3·301$
	—6	39·2500	38·5000	0·7500	$df = 11$
	$F_2\,ex$ **BB** $\times$ **+**—1	36·5400	37·1200	−0·5800	$P < 0·01$
	—2	39·0500	38·5000	0·5550	
	—3	38·5500	38·3500	0·2000	
	—4	37·6000	35·6500	1·9500	
	—5	40·0000	38·4167	1·5833	
	—6	37·3500	36·4500	0·9000	
Females:	$F_2\,ex$**+** $\times$ **BB**—1	46·0000	45·2000	0·8000	$\bar{d} = 0·8238$
	—2	44·7690	44·7500	0·0190	$V_d = 0·5171$
	—3	44·8000	43·8000	1·0000	$V_{\bar{d}} = 0·08619$
	—4	46·4000	44·3750	2·0250	$s_{\bar{d}} = 0·2936$
	—5	44·4000	43·4550	0·9450	$t = 2·806$
	—6	44·5830	44·4290	0·1540	$df = 6$
					$P = 0·05–0·02$

and must be reserved for later discussion. The actual F_3's used in the selection experiments were chosen from this large number of random mated cultures. The three with the highest sum of parental hair numbers were taken to start the high line and the correspondingly lowest three to start the low line. Subsequently the method of selection and mating was exactly the same as that described for the cross $\underline{y} \times$ **f** $\mathbf{B^i B^i}$ except that three matings were set up in each selected line in every generation, all giving progeny being counted and used for the next selection. Table 3 and Text-fig. 4 show the results of selection in this cross. Text-fig. 3 shows the results of plotting the difference between high and low line against generations, exactly as for $\underline{y} \times$ **f** $\mathbf{B^i B^i}$. It is less subject to environmental fluctuation and so easier to read than is Text-fig. 4.

(b) *Results*

The striking feature of the results from this cross is that after an initial response to selection for two generations, stability is attained exactly as in $\underline{y} \times f\,B^i B^i$, but this period of stability is itself succeeded, in both high and low lines, by an even more marked advance in the direction of selection than that which occurred in the first place. The second advance persisted for several generations before a second stable level was reached. It is to be regretted that such marked sterility had appeared in the selected lines that it was impossible to carry the experiment on after thirteen generations. So it is impossible to say whether a third advance would have occurred at a later point.

The second advance in the direction of selection had much greater results than did the first one. It led to the production of lines with more and less hairs, respectively, than have ever been seen in any stock culture, except for those which carry known major mutations affecting hair number, such as **sc**. The selected lines had gone outside the limits of the normal wild or cultured populations.

It was the second advance which produced this enormous selective effect. The first change with selection is most reasonably interpreted as due to recombinations of whole chromosomes present in the hybrid, as in the case of $\underline{y} \times f\,B^i B^i$. That such a change with selection is to be expected follows from the demonstration that the genes on the X chromosome of **BB** are on the whole plus genes as compared with those of Oregon **+**, and its corollary that the **BB** autosomes are preponderantly minus in effect relative to the Oregon stock. Thus assortment of chromosomes in the hybrid should lead to an initial advance under the action of selection. The most favourable combinations will, however, soon be sorted out and stability will follow.

The second and larger advance cannot be accounted for in this way; it must depend on the presence of a different type of heritable variation. There seem to be two obvious ways in which such variations could arise, by mutation and by recombination of genes within chromosomes. Careful consideration must however lead to the conclusion that mutation is an unlikely origin. The advance occurred simultaneously in both lines and was of a magnitude greater than that ascribable to recombination of whole chromosomes. Thus the necessary mutation would be of frequent occurrence and having an effect of a greater magnitude than seems reasonable from what we know of the process. Recombination within the chromosomes is a much more plausible explanation. Intra-chromosome

recombination is an inevitable occurrence, but to be effective in releasing variation it must lead to a marked redistribution of the plus and minus genes. The way in which such a reorganization could take place is shown in Text-fig. 5. In Text-fig. 5 A a simple single cross-over would lead to the desired effect; but in the situations of 5 B and 5 C more complex recombination would be necessary to produce an equivalent reorganization. The chief point to note is, however, that for recombination to produce the necessary variation, the chromosome must originally have carried polygenic combinations which were more or less balanced, i.e. which had more or less equal numbers of plus and minus genes. Recombination thus leads to unbalancing of the combinations by producing an excessive
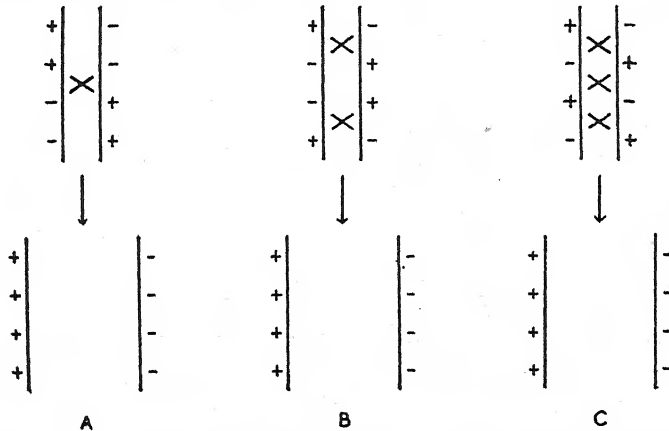
Text-fig. 5. Diagram to illustrate how balanced polygenic combinations may release variation by recombination. As the intermingling of the genes becomes more complex, higher order crossing-over becomes necessary for the full release of the potential variation; some of it may, however, be released by lower-order crossing-over.

number of plus modifiers on one and minus modifiers on the other derived chromosome. The origin of such balanced polygenic combinations will be discussed later.

The hypothesis that the second advance was due to the release of variation by recombination and not by mutation was tested in three ways. These were: (a) to select within the Oregon+ line, which being highly inbred could show variations only as a result of mutation and so provides a measure of the mutation rate; (b) by repeating the **BB** × **+** selection experiment, which would not be expected to give the same result if the relatively uncommon process of mutation were the cause but which should show the same behaviour if the relatively frequent occurrence of recombination released the variation; and (c) by following

the effects of selection in lines in which heterozygous females, showing recombination, and males, which fail to show recombination, respectively were continually backcrossed to a homozygous stock. The results of these tests are described in the next three sections.

Before leaving the **BB** × **+** cross, it may be added that after the high and low lines had been selected for thirteen and twelve generations respectively, they were mass cultured without selection and later inter-crossed reciprocally (Table 5). Two features of interest were observed in

<div align="center">Table 5</div>

		Original cross		Intercross of selected lines	
		Mean	Variance	Mean	Variance
BB Parent:	Males	36·06	6·0065	43·10	4·1667
	Females	43·48	8·2708	49·13	7·1137
+ Parent:	Males	39·88	6·5922	29·91	6·2909
	Females	44·59	9·0700	35·79	12·7310
F_1:	Males	37·83	5·7710	35·86	6·6869
	Females	43·40	4·9080	40·34	8·3389
F_2:	Males	38·35	6·8201	37·92	16·8555
	Females	44·20	7·0436	42·80	18·0826

The means and variances given are the average values of all the appropriate cultures.

the F_2 of these crosses. In the first place the variance of these F_2's was much greater than that of the original F_2 from **BB** × **+** (Table 5) as would be expected if released variation had been picked up and fixed in the course of selection. Secondly the segregation of the X-chromosome could be followed in this cross, as the high selection line was homozygous **BB** and the low line **+**. In the F_2 the difference between the **BB** and **+** flies was determined as described for the original cross. The average difference between **BB** and **+** males in the first five cultures was 0·8749, which is to be compared with a value of 1·1350 in the original F_2. Thus there is no evidence that the gene content of the sex chromosome has been affected by selection. Whatever produced the change which led to the second advance it must have occurred in one or more of the auto-somes.

5. Selection in the Parental Lines

(a) *Oregon* **+** : *Technical*

The method of selection practised in the Oregon **+** line was just the same as that used in the cross-bred material. At the time when selection commenced the line had been kept by brother-sister mating for at least seventy generations, and almost certainly had been inbred for a con-siderable time before the available records began. Hence it should be homozygous except for the effect of mutation.

A single culture was used to commence the experiment. The single pair matings were made in each of the two selection lines, high and low. In the former case the matings were between the highest three males and females, and in the latter case between the lowest three of each sex. In each generation the highest three males and females in the high line were chosen as parents of the three cultures of the next generation, and correspondingly in the low line. Occasional departures were made in that sometimes a slightly lower, in the high line, or higher, in the low line, individual would be taken in order to keep all the cultures represented in the next generation. The matings were made between flies from different cultures of the same line as far as possible, just as in the crossbred material of the previous sections.

(b) Oregon + : Results

The results of the selection are given in Table 6 and shown graphically in Text-fig. 6. It will be seen that selection had no effect in the twelve
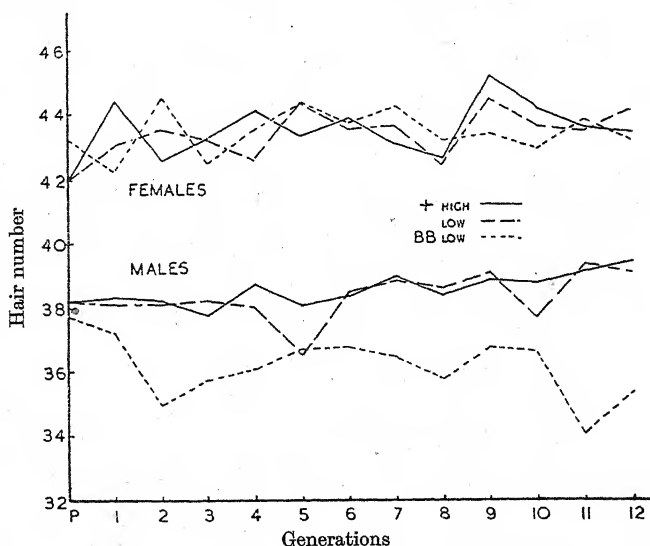
Text-fig. 6. Selection in the Oregon+ and **BB** lines. Only the means of each generation are shown. (See also Text-fig. 3.)

generations for which it was practised. This is shown even more strikingly in Text-fig. 3 where, as in the previous cases, the mean difference between high and low lines is plotted against generations. This difference is as often negative as positive, i.e. the low line frequently has a higher hair number than the high line.

Table 6 A. *Oregon+ line*

Generation	Males No. counted	Males Mean	Females No. counted	Females Mean	Generation	Males No. counted	Males Mean	Females No. counted	Females Mean
Unselected	17	38·24	20	42·00	L 1—1	20	37·70	20	43·45
H 1—1	20	38·40	17	44·41	—2	20	38·50	20	43·00
—2	20	38·35-	20	44·50	—3	20	38·30	20	42·80
H 2—1	20	38·40	14	42·71	L 2—1	20	37·55	20	43·80
—2	20	38·25	20	42·80	—2	20	38·55	20	43·90
—3	20	37·85	20	42·40	—3	19	38·61	20	43·45
H 3—1	20	38·15	18	43·17	L 3—1	20	38·70	20	42·85
—2	20	37·40	20	43·50	—2	20	38·20	20	43·65
—3	20	37·65	20	42·95	L 4—1	18	38·05	20	43·05
H 4—1	20	39·20	20	43·50	—2	20	37·95	20	42·30
—2	20	38·05	20	44·50	L 5—1	20	36·50	20	45·05
—3	20	38·90	20	45·05	—2	20	37·00	20	44·50
H 5—1	20	37·05	20	42·55	—3	20	36·10	20	43·50
—2	20	39·00	20	44·20	L 6—1	20	38·00	16	43·13
H 6—1	20	38·60	20	43·55	—2	20	39·15	20	43·90
—2	12	38·00	14	43·57	—3	20	38·35	20	43·55
—3	20	38·50	20	44·65	L 7—1	20	38·35	20	43·95
H 7—1	9	39·44	3	44·00	—2	20	40·30	20	44·05
—2	20	38·35	20	43·15	—3	20	37·95	20	42·80
—3	20	39·10	20	42·95	L 8—1	20	38·80	20	42·65
H 8—1	20	38·30	20	43·25	—2	20	38·70	20	42·60
—2	20	38·50	20	42·05	—3	20	38·45	20	41·95
H 9—1	20	38·55	20	45·35	L 9—1	20	40·00	20	44·85
—2	20	39·15	20	43·85	—2	20	38·80	20	45·10
—3	20	39·00	20	43·00	—3	20	38·60	20	43·50
H 10—1	20	38·35	20	43·60	L 10—1	20	37·30	20	43·90
—2	20	39·45	20	44·55	—2	19	38·05	6	41·83
—3	20	38·50	20	44·35	—3	20	38·15	20	43·20
H 11—1	20	39·55	20	42·95	L 11—1	20	39·10	20	44·55
—2	20	39·00	20	43·80	—2	20	40·05	20	43·45
—3	20	38·80	20	43·80	—3	20	39·20	15	43·20
H 12—1	20	39·80	20	42·90	L 12—1	20	39·50	20	44·35
—2	20	39·10	20	44·05	—2	20	39·65	20	43·70
					—3	20	38·30	20	44·45

Table 6 B. **BB** *line*

Generation	Males No. counted	Males Mean	Females No. counted	Females Mean	Generation	Males No. counted	Males Mean	Females No. counted	Females Mean
Unselected	8	37·75	4	43·25	L —2	10	35·40	8	44·88
L 1—1	20	34·90	19	41·10	—3	10	37·30	6	43·33
—2	8	37·63	11	43·55	L 8—1	6	35·00	11	43·27
L 2—1	9	35·00	6	44·50	—2	20	36·40	17	43·06
L 3—1	11	36·09	9	42·33	L 9—1	14	37·36	6	42·83
—2	9	35·33	11	42·82	—2	9	37·33	9	43·33
L 4—1	20	36·10	20	43·60	—3	20	35·85	20	44·05
L 5—1	20	36·45	18	44·27	L 10—1	16	36·63	10	42·90
—2	15	37·00	17	44·65	L 11—1	20	34·10	14	44·29
L 6—1	14	37·50	6	43·80	—2	16	33·94	16	43·25
—2	17	36·88	10	43·10	L 12—1	10	36·90	5	44·80
L 7—1	20	36·60	14	44·57	—2	8	33·75	7	41·29

Nomenclature as in Table 2.

Selection in this inbred line is thus ineffective, or in other words, mutation does not lead to the release of any perceptible amount of genetic variation over a period of twelve generations. Thus the second advance in the crossbred **BB** × **+** lines cannot be due to mutation. This conclusion is subject to the proviso that hybridization cannot raise the mutation rate sufficiently to account for the difference between the inbred and the outbred material. Sturtevant (1939) has indeed reported that in inter-racial crosses of *D. pseudoobscura* the lethal mutation rate is increased as compared with that of the pure races. But even in such wide crosses as these the increase in mutation rate would hardly be sufficiently great to warrant the conclusion that the second advance in **BB** × **+** could be due to mutation, when the mutation rate is imperceptible in the inbred line. The advance must be due to intra-chromosome recombination.

(c) **BB**

An attempt was made to select high and low lines from the **BB** parental stock. The high line died out very quickly owing to sterility, but the low line was maintained, though often precariously, for sixteen generations, mainly by single-pair matings, though occasionally by mass mating. Table 6 and Text-fig. 6 show the results of the first twelve generations of this experiment. The last four were so small as to be worthless. In spite of the **BB** stock's not being inbred, there was no marked response to selection in the females, though the males changed slightly. This strengthens the conclusion that the second advance in the hybrid material was due to recombination leading to unbalance of polygenic combinations within the chromosomes.

6. The second **BB** × **+** experiment

(a) *Technical*

This selection experiment was conducted on the same general lines as its predecessor, but differed in some technical details. The initial cross was made reciprocally, using three females each way. The flies were allowed to lay in the bottles for about 5 days. The lengthened laying period was adopted throughout the whole of this experiment. It may perhaps have led to some reduction in hair number from overcrowding, but this will have been constant throughout the experiment and more especially will have been operative in both high and low lines. Hence it will be eliminated as an environmental discrepancy when the difference between these two selected lines is plotted against generations.

Two F_2's were raised from each of the reciprocal F_1's. In these the segregation of **BB** and **+** flies could be observed and, as before, their difference in hair number estimated. It proved to be 0·9501. This is slightly, certainly not significantly, less than in the first F_2. It is, however, remarkable that whereas in the first cross the high line soon became homozygous **BB** and the low line **+**, this had not happened after seven generations in the second experiment. Such behaviour is perhaps a consequence of the modified system of mating.

The F_3 generation was composed of two high and two low cultures. Each culture comprised the offspring of two selected males and two selected females mated altogether in one tube. The subsequent generations were made up in the same way, the parents of the high line cultures being the highest flies from the previous generation of that line, and similarly for the low line.

(b) *Results*

The results are shown in Table 7 and Text-figs. 3 and 7. The graph in Text-fig. 3 is especially instructive as, being the mean difference between the two selected lines, it is largely free from environmental discrepancies. It confirms the main behaviour of the first **BB** × **+** selection experiment in that, after a period when a response to selection is absent, a marked advance suddenly sets in. It differs from the first cross in showing little if any initial rise prior to the time of stability which itself finishes at the third generation. This is perhaps not so remarkable, as the **BB** stock was known to be inhomogeneous for hair number polygenes (§ 2). It is, however, clear that the advance following stability is a characteristic of the behaviour of this material under the action of selection; and in consequence is unlikely to be due to mutation. Recombination by crossing-over, which is a regular feature of chromosome behaviour in the female is thus a much more likely explanation.

7. The backcross lines

(a) *Technical*

A direct test of the recombination hypothesis was made commencing with the F_1 of the second **BB** × **+** cross, as described in the previous section. Two cultures both containing F_1 females mated with two Oregon **+** males, were set up. Two further crosses, each utilizing two F_1 males and two Oregon **+** females, were also made. These were termed respectively the female and male lines. In each line selection in both high and low directions was practised, beginning with the progeny of this

Table 7. *The second* **BB** × + *experiment*

Generation	Males No. counted	Mean	Females No. counted	Mean	Generation	Males No. counted	Mean	Females No. counted	Mean
F_1 + ×**BB**	15	36·47	20	41·00	H 8—1	20	39·90	20	45·75
BB × +	20	37·30	15	41·20	—2	20	39·45	20	45·65
F_2 —1	20	34·55	20	39·50	L 1—1	20	35·65	20	42·85
ex + ×**BB**—2	20	34·45	20	39·85	—2	20	34·20	20	40·20
ex **BB** × +—1	20	34·80	20	40·10	L 2—1	20	35·25	20	40·10
—2	20	35·90	20	40·90	—2	20	35·15	20	39·75
H 1—1	20	37·00	20	41·10	L 3—1	20	32·20	20	38·90
—2	20	35·30	20	41·05	—2	20	33·20	20	37·90
H 2—1	20	35·00	20	41·80	L 4—1	20	33·60	20	39·00
—2	20	35·05	20	42·05	—2	20	31·65	20	39·05
H 3—1	20	37·70	20	43·20	L 5—1	20	30·65	20	37·35
—2	20	38·00	20	41·30	—2	20	32·53	20	38·65
H 4—1	20	39·10	20	44·60	L 6—1	20	32·80	20	38·40
—2	20	36·95	20	44·15	—2	20	31·95	20	38·25
H 5—1	20	37·85	20	44·10	L 7—1	20	30·75	20	36·70
—2	20	37·35	20	44·80	—2	20	31·60	13	36·23
H 6—1	20	40·35	20	46·35	L 8—1	20	30·25	20	35·95
—2	20	38·65	20	45·10	—2	18	29·72	20	35·35
H 7—1	20	40·15	20	45·95					
—2	20	39·85	20	44·75					

Nomenclature as in Table 2.
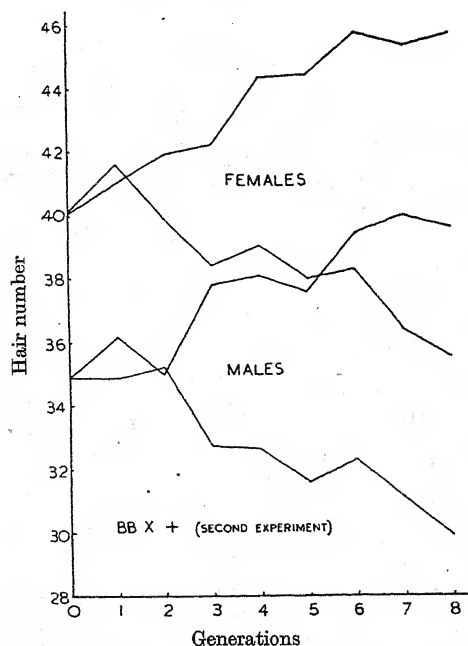
Text-fig. 7. The effect of selection in the second **BB** × + experiment. (See also Text-fig. 3.)

first cross. Each new generation in the female high line was composed of two cultures each having two female parents which were the high selections from the previous generation of the high line and two male parents which were inbred Oregon +. The female low line was similarly kept with low selections. The two male lines differed in that selected males were used to mate to inbred females. The four lines were kept quite separately.

(b) *Results*

Now the males differ from females in showing no recombination. Hence any advance in the male lines should be made immediately, stability or regression due to backcrossing following later. The female lines could, on the other hand, still show a delayed but larger advance due to recombination. There are, however, certain difficulties inherent in the experiment, notably that the rate of regression in hair number towards the Oregon + value, consequent on the continued backcrossing to that line, will differ in the two lines as a result of the difference in recombination. It should be noted also that in these lines selection will be effective only by action on dominant or partially dominant genes from the **BB** line and so may not have the same results as in previous experiments. Thus this experiment, which at first seems so suitable for testing the recombination hypothesis, may not in fact be straight-forward.

The results obtained (Table 8 and Text-figs. 8, 9) bear out this expectation. In general they support the recombination view. From Text-fig. 9 it will be seen that the male line never, after the first generation, showed so marked an advance as the female line, and had regressed much more sharply at the end of seven generations. One disconcerting result is, however, apparent: that the male line shows a marked advance between the third and fifth generations, when the female line is also advancing rapidly. A closer analysis of the data (Text-fig. 8) helps to clarify this. In both male and female crosses one line fails to react to selection, viz. the low female line and the high male line. Now the low female line almost immediately attains the hair number of Oregon +, as shown by comparison with the inbred material of that stock. Hence this line had most probably become effectively homozygous Oregon + before selection began to act. Thus the difference between the high and the low female lines, as plotted in Text-fig. 9, is a measure of the delayed advance in the high line.

The story is, however, somewhat different in the male lines. Here the high selected line failed to show any material response. But it stayed *below* the Oregon + value for four generations. The cause of such be-

Table 8. *The backcross lines*

Parental and F_1 generations as in Table 7

	Males		Females	
	No.		No.	
Generation	counted	Mean	counted	Mean
♀ O—1	20	36·25	20	42·25
—2	20	35·90	20	41·20
—3	20	37·00	20	42·50
—4	18	37·28	20	42·95
♀ H 1—1	20	37·60	20	41·95
—2	20	36·90	20	41·50
♀ H 2—1	20	39·45	20	46·40
—2	20	38·55	20	43·80
♀ H 3—1	20	39·95	20	44·30
—2	20	38·65	20	44·45
♀ H 4—1	18	41·44	20	46·00
—2	20	39·55	20	45·10
♀ H 5—1	7	44·43	7	49·71
—2	20	39·20	20	42·80
♀ H 6—1	20	41·05	20	46·80
—2	20	40·25	20	46·00
♀ H 7—1	12	41·25	20	47·35
—2	17	42·76	15	47·93
♀ H 8—1	15	40·87	20	46·35
♀ L 1—1	20	36·55	20	41·70
—2	20	37·25	20	41·85
♀ L 2—1	20	38·40	20	43·75
—2	20	37·40	20	41·15
♀ L 3—1	20	38·40	20	43·40
—2	20	38·25	20	43·20
♀ L 4—1	20	38·65	20	44·26
—2	20	37·30	20	41·80
♀ L 5—1	20	38·75	20	42·80
—2	20	37·65	20	42·15
♀ L 6—1	20	38·40	20	42·50
—2	20	38·80	20	43·80
♀ L 7—1	20	38·60	20	43·70
—2	20	38·85	20	43·85
♀ L 8—1	20	38·50	20	37·55
—2	20	43·00	20	42·65

	Males		Females	
	No.		No.	
Generation	counted	Mean	counted	Mean
♂ O —1	20	37·60	20	42·75
—2	20	36·80	20	41·85
—3	20	37·60	20	42·10
—4	20	36·85	20	42·50
♂ H 1—1	20	38·75	20	42·10
—2	20	38·10	20	42·50
♂ H 2—1	20	38·60	20	41·95
—2	20	38·50	20	41·50
♂ H 3—1	20	37·85	20	41·90
—2	20	38·25	20	42·70
♂ H 4—1	20	38·70	20	43·30
—2	20	38·35	20	44·25
♂ H 5—1	20	38·20	20	43·05
—2	20	39·30	20	44·15
♂ H 6—1	20	38·60	20	44·10
—2	20	37·80	20	42·95
♂ H 7—1	20	38·10	20	44·30
—2	20	37·35	20	44·95
♂ H 8—1	20	36·90	20	42·60
—2	20	38·50	20	42·90
♂ L 1—1	20	36·20	20	42·80
—2	20	38·30	20	42·50
♂ L 2—1	20	39·75	20	43·05
—2	20	36·85	20	41·20
♂ L 3—1	20	36·35	20	40·55
—2	20	37·35	20	42·15
♂ L 4—1	20	38·40	20	41·65
—2	20	36·90	20	41·80
♂ L 5—1	20	36·10	20	40·90
—2	20	36·65	20	39·70
♂ L 6—1	20	36·05	20	42·05
♂ L 7—1	20	38·25	20	43·25
—2	12	39·25	12	45·33
♂ L 8—1	20	37·05	20	41·35
—2	20	42·30	15	37·07

♀ = line in which selected females were crossed to Oregon + males.
♂ = line in which selected males were crossed to Oregon + females.

The generation O is the offspring from the backcross of F_1 to Oregon + and so has no ancestry of selection.

Other nomenclature as in Table 2.

haviour is a complete mystery. When backcrossing a lower line to Oregon + and selecting for high hair number the Oregon + value should on any view be rapidly attained by the choice of homozygotes. This,

however, failed to happen, and when after four or five generations this high line behaved as expected and reached the Oregon + level, its effect was to show an apparently delayed advance with selection when compared with the male low line, which had in the meantime been stable
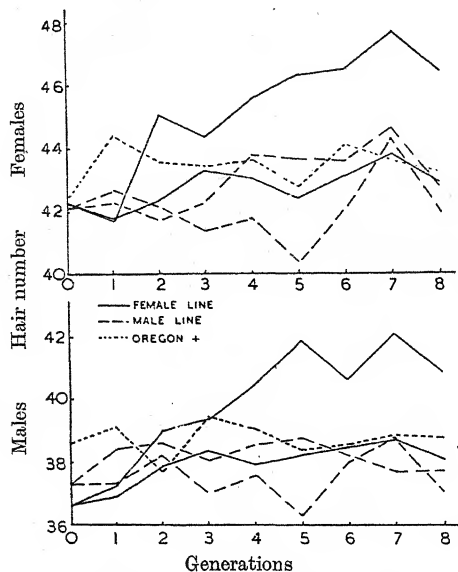
Text-fig. 8. The effects of selection in the male and female backcross lines. The "control" line is that of the low selection in the inbred Oregon + stock.
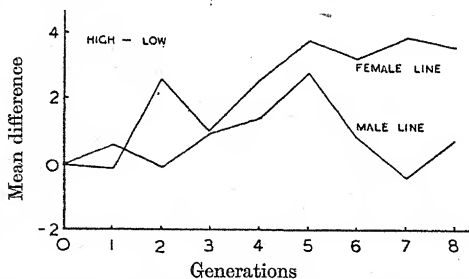
Text-fig. 9. The mean differences between high and low selection in the male and female backcross lines.

with regard to Oregon +. Thus the delayed advance of the male line is not due to the same cause as that of the female lines. Rather it should have been apparent several generations earlier, on the recombination as on any other view. So, on the whole, these backcross selection experiments support the recombination view, though the results have been partially obscured by some inexplicable extraneous factor.

8. The development of polygenic combinations

It seems clear from the experimental evidence that strains of *D. melanogaster* can differ by balanced polygenic combinations within chromosomes. These combinations have approximately the same effect as each other on hair number, but recombination following crossing-over between them results in marked upset of the balance and in the release of considerable variation on which selection can act.

Having shown that balanced combinations occur, the next question which arises is that of how such balanced combinations come into being. Some opinion of their origin can be formed from a comparative consideration of the various selection experiments which have been conducted using *D. melanogaster* as material. These fall into two groups, those concerned with selection for naturally variable characters and those in which selection was exercised on the expression of a mutant character. The experiments described above fall into the first group, as hair number is now known to be genetically variable in wild flies. One other large experiment is in this class too, viz. Payne's (1918) selection for extra scutellar bristles. Such extra bristles are found as a rarity both in culture and in wild material. Selection for increased scutellars had little effect for several generations, but this stability was followed by a sharp advance, just as in the present hair number selections. Jumps and stability alternated for a considerable time in Payne's flies. His mating system is not clearly described, but it seems quite likely that even after thirty generations the degree of inbreeding in his material was quite small, so that recombinations could still be effective. Thus his results agree with my own in two important aspects, viz. (*a*) early stability was followed by a marked response to selection, and (*b*) the final result of selection was to give flies showing a degree of expression of the character well outside the range observable in the material from which the experiments started. Both these results would follow from the breaking-up of balanced polygenic combinations by crossing-over.

The second group of selection experiment, that concerned with the manifestation of mutant characters, shows results in marked contrast to those mentioned above. Three main pieces of research seem to fall in this class, viz. selection for (*a*) bristle number in Dichaete flies (Sturtevant, 1918), (*b*) bristle number in a stock homozygous for a recessive bristle increaser (MacDowell, 1917), (*c*) facet number in Bar (Zeleny, 1922; Zeleny & Mattoon, 1915). In each case a large advance in the direction of selection was achieved at the beginning of the experiment, and there

was no evidence of marked delayed advances. Furthermore, in each case there was no marked transgression of the original limits of the character in the later generations. Thus these results would be expected if balanced polygenic combinations did not exist in the original material.

The position of Payne's (1920) later experiments on selection for the number of bristles in scute flies is obscure. One of his lines seems to show delayed and the other immediate response to selection. This experiment will require repetition before it can be classified.

So, on reviewing the evidence, it appears that balanced polygenic combinations are encountered when naturally variable characters are
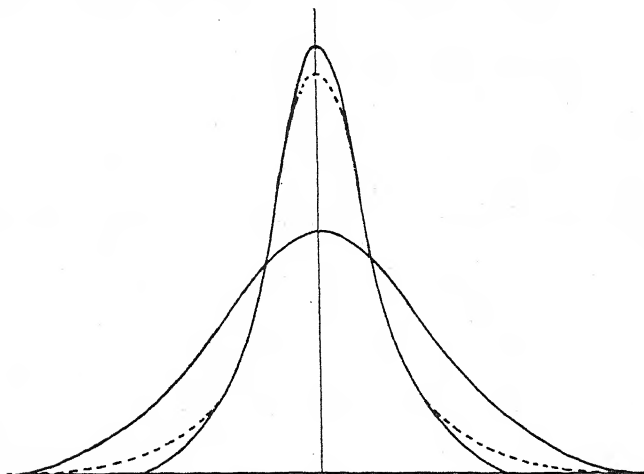
Text-fig. 10. Diagram to illustrate the relation between polygenic variation and linkage. If the outer full curve is that of variation when the genes are unlinked, and the inner full curve that obtained when variability is lowered by reduction in the number of segregating genes, the dotted curve is that obtained when variability is reduced by linkage. Marked variability reduction is obtained, but the two extremes of the curve are still as wide as they were originally.

used, but that the modifiers of expression of mutants are not so organized. This clearly suggests that the balanced combinations are themselves the product of natural selection, which would be relatively inoperative on the modifiers of very rare mutants.

In order to see how natural selection would bring about balanced combinations we must consider the effect of linkage on variation in the wild. Text-fig. 10 shows a series of frequency distribution curves of the kind characteristic of polygenic characters as found in natural material. Most of the individuals show expressions of the character near the mean, and as the degree of expression becomes more remote from the mean the frequencies of individuals showing it become less. In order to simplify

our argument let us suppose that the curve is symmetrical and let us consider at present only the genetical variation for the character. The conclusions reached are not invalidated by skewness, and environmental fluctuations merely reduce the efficiency of selection. Hence our model is in no wise deceptive. Now such curves have very definite selective implications. Of these the most important is that the mean of the observed distribution is near to the optimum value for the environment under consideration, the individuals with the more extreme high and low values being at a selective disadvantage. If this were not so and if the optimum were, say, markedly higher than the mean the individuals near the optimum would leave more descendants than those near the mean and this would lead to the piling up of numbers near the optimum to which, in a few generations, the mean would closely approximate. Thus the mean and the optimum will be near to one another, but they will never quite coincide, as the environment, and hence the optimum, is constantly changing slightly. The mean fluctuates with the optimum, though less widely, and always one generation in arrears.

Now if the selective disadvantage of an individual increases as its degree of character expression deviates more and more markedly from the mean, the very occurrence of genetical variation implies a reduction in the average fitness of the progeny of an individual. For the mean variability of the progeny of an individual will be highly correlated with the variability of the population as a whole, except in those rare cases where the population consists of a series of pure lines. Hence genetical variation round the mean is disadvantageous. Then one effect of natural selection will be to reduce this variability.

It is equally clear, however, that the variation cannot be wholly eliminated without danger to survival through failure to react to the changes in the environment, such as must be constantly occurring. Such invariable individuals would be at a disadvantage as compared with their more variable competitors.[1] Thus the organism is faced with a paradoxical situation in which variation results in an immediate loss of fitness while lack of variation means deferred but none the less serious disadvantage. In general then long term selection will effect a compromise between the two extremes. The mechanism of this compromise is linkage leading to balanced polygenic combinations.

[1] Environmental changes may be divided into two classes. There are directional trends continuing over long periods to which the organism must adapt itself or perish. There are also non-directional fluctuations to which the organism need not respond. In the case of ephemerals the seasonal changes are important in that the organism must not react strongly to the environmental cycle.

The way in which selection will act to build up these combinations can be seen from a simple example. Let us suppose that we have a population heterozygous for two genes (or groups of genes) **A** and **B**, cumulative in action, each of which is without dominance, and having gene frequencies of $\frac{1}{2}$. Thus **Aa** is intermediate between **AA** and **aa**, and **AaBb** will show the character in question to the same degree as **AAbb** and **aaBB**. Then we have nine genotypes which fall into five phenotypic groups, as follows:

2	**AABB**
1	**AaBB, AABb**
0	**AAbb, aaBB, AaBb**
−1	**Aabb, aaBb**
−2	**aabb**

The central group will be at the mean of the distribution and hence near the optimum. Now **AABB** and **aabb** are at a marked disadvantage as compared with the central class and **AaBB, AABb, Aabb** and **aaBb** are also at a disadvantage through not so great a one.

This means that selection will gradually reduce the numbers of **AB** and **ab** gametes produced as compared with the numbers of **Ab** and **aB** types. But **AB** and **ab** gametes are being continually replenished from the double heterozygotes which are formed from the fusion of **Ab** and **aB** gametes. Thus the action of selection in reducing variation and increasing immediate fitness is slowed down by random recombination of the two genes. If, however, **A** and **B** are linked this replenishment is reduced in rate and the number of zygotes in the disadvantageous classes, notably **AABB** and **aabb**, thereby diminished. So linkage carries an advantage in increasing the fitness of the population as a whole and selection will favour reduced recombination in doubly heterozygous individuals and hence ultimately in the population as a whole.

It is however important to note that although the proportion of **AB** and **ab** gametes is reduced by linkage in this way, some gametes of these types will be formed so long as any recombination does occur. Then if the environment changes in such a way as to cause a marked change in the optimum the corresponding change in the genetic constitution of the population can and will occur by means of the recombination. If **AB** or **ab** types are now favoured they are present and can be selected as a direct consequence of the action of crossing-over. Thus linkage of **A** and **B** results in the storage of variability in such a way that the immediate fitness of the population is maintained at a high level, while

still gradually releasing variation, which makes possible a response to change in environment. **Ab** and **aB** are in fact balanced combinations.

Other causes of reduction of variability, such as homozygosity for **Ab** and **aB**, will be favoured immediately, but will eventually become disadvantageous when the environment changes. Such homozygosity must occur to some extent in nature. At this point it is important to remember that natural variation is preponderantly polygenic. In our simple illustration such homozygosity for one factor would change the whole set-up, but in the case of a polygenic character, dependent on possibly scores of genes, a much less erratic mean behaviour is expected. Some genes may become homozygous, but the remainder will still remain heterozygous and act in balanced combinations. Probably the proportions falling into the two classes will be a definite property of the genic organization and mutation rate of the species. Furthermore, in the simple digenic case considered above, only a proportion of the individuals were doubly heterozygous, and so open to the action of selection on recombination. With a polygenic character this proportion will be much higher and the action of selection correspondingly greater.

Thus it will be seen that, granted the occurrence of crossing-over and of some measure of outbreeding, the origin and behaviour of balanced combinations is an inevitable consequence of natural selection in a slowly changing environment. Heterozygosity for such combinations is the only mechanism which allows of maintenance of the variability necessary for future change in such a way that it does not constitute an immediate drawback. As such combinations are dependent for their working on linkage and recombination it is clear that this mechanism also allows one to see the real value of crossing-over to the organism. Such selective control of linkage is essentially similar to that postulated by Sturtevant & Mather (1939) but whereas they considered linkage of two genes, with its concomitant difficulties of maintenance of heterozygosity, it is now seen that the process depends on polygenic characters and so this difficulty vanishes.

9. THE INTERACTION OF POLYGENIC COMBINATIONS

Balanced polygenic combinations can act as storing mechanisms for variation only if at least two occur in various representatives of one chromosome, i.e. only when some heterozygosity, real or potential, is present. Actually many more than two will exist.

Now any combination is balanced in that it contains + and − genes in a proportion which makes it, in combination with itself and the other

combinations in the population, suitable to the environment. The fact that such a combination may be homozygous clearly implies close internal balancing if it is to survive. On the other hand, the fact that two combinations may occur together in one zygote means that they must be balanced one against the other so that their heterozygote is fitted for the environment. Thus two individuals, homozygous for different polygenic combinations, might both fall near the optimum in the variation range, yet their heterozygote might display heterosis, i.e. express the character to a markedly different and hence less favourable degree. In this case the heterozygote is at a disadvantage as compared with the homozygote and any mechanism tending to prevent inter-crossing of the homozygotes will be favoured. Heterosis in such cases is disadvantageous and its occurrence will favour the origin of an isolation mechanism. Since heterogeneity for polygenic combinations is almost certain within populations, and hence inevitable between populations, it seems extremely likely that this may be a widespread cause of genetical isolation mechanisms (cf. Sturtevant, 1940). The effect of heterosis must however be clarified by further knowledge of the dominance relations of polygenes, before a final decision on its importance in this connexion can be reached.

Thus there are two balancing processes to which any polygenic com-bination is subject, (a) the internal one in homozygotes and (b) the relational balancing against other combinations. Both will occur in any population, but their relative effectiveness may vary according to the breeding behaviour of the organism. Where inbreeding is common, the opportunity for relational balancing will be rare and we may expect selection to act mainly on the internal constitution of the combinations. In such cases heterozygotes may be less well adapted than homozygotes and outcrossing will tend to be discouraged. The stored variability of the population then lies in its potential heterozygosity.

Where outcrossing is regular the occurrence of homozygotes may be rare and the relational balancing process becomes the more important. Good internal balance may then fail to be achieved and homozygotes will be poorly adapted. This will favour more certain outcrossing. Thus the breeding mechanism, whether in- or out-breeding in type, will tend to be strengthened in its action. It is not clear how far-reaching this process will be, and discussion is difficult without further knowledge of the organization of balanced combinations. It seems, however, highly probable that enforced hybridity of the type found in *Oenothera* and in the sex mechanism may have developed along these lines.

10. POLYGENIC COMBINATIONS AND POLYMORPHISM

A considerable number of cases are known where single gene characters regularly show heterogeneity in the wild. Species showing this behaviour are termed polymorphic. The occurrence of such regular polymorphism immediately raises the question of its maintenance; for normally if one allelomorph of a gene has a selective advantage over another the latter almost vanishes, its frequency becoming of the order of its mutation rate. Certain properties of polymorphism genes have been established which enable us to understand their occurrence better. Thus Fisher (1930), using the data of Nabours (1925), has shown in *Apotettix* that individuals heterozygous for these genes are more viable than their homozygous sibs. Since, however, this must be true of all such heterozygotes, if it is to provide an explanation for the existing polymorphism, it would seem unlikely that the viability differences are properties solely of the genes determining the visible polymorphism. It would seem more likely to be dependent on the complete linkage of these genes with, or their occurrence in, polygenic combinations, which had attained a particular relational balance.

As has been seen above, where outcrossing is the rule the relational balance may be more carefully adapted by selection than the internal balance. Then the homozygotes will show the character too much or too little and would be at a disadvantage as compared with the heterozygotes. This should tend to encourage mechanisms favouring outcrossing and in consequence we might expect a polymorphic animal such as *Apotettix* to show a preference for mating of unlike types. Unfortunately, no data are yet available for the verification of this point.

The question of the existence of various chromosome sequences ("inversions") in wild *Drosophila* probably falls into this category too. In fact, Sturtevant & Mather (1939) have suggested that such heterogeneity depends on interaction between the genic contents of such structurally variable chromosomes. It is notable that their argument, as translated into our present terminology, implies a better relational than internal balance, just as in *Apotettix*. In fact if each sequence in the third chromosome of *Drosophila pseudoobscura* carried a dominant marker gene, this species would simulate the behaviour of the grouse locusts. The structural variation in *D. pseudoobscura* is almost completely confined to one chromosome, viz. the third. It seems reasonable to expect that, in general, polygenic combinations will not be evenly distributed amongst all the chromosomes of the complement. The mechanism of

variability storage is such that a new combination will frequently be more effective when in the same chromosome as those already existing, and so the *D. pseudoobscura* mechanism should often arise.

In a cross-breeding population any combination will occur most frequently heterozygous with other combinations in that same chromosome. Since there will be a number of such other combinations the selective advantage or disadvantage of the particular one in question seems likely to be capable of very delicate adjustment as a result of change in its frequency. Thus a combination when occurring in 10 % or 50 % of the gametes of a population in a given environment may be at a disadvantage, but when its frequency is say 30 % of the gametes it may be properly adjusted. In a somewhat different environment perhaps 10 % would be the proper frequency. That this will be so cannot be stated definitely until a full mathematical analysis has been undertaken, but it seems very likely to be true.

Granted the validity of this supposition, a very simple explanation of Huxley's "clines" becomes possible. Thus populations of Guillemots regularly contain a number of so-called bridled birds in addition to normal individuals. The frequency of bridling changes more or less regularly with the geographical location of the populations. Now if the bridling allelomorph is completely linked to one polygenic combination, just such a cline would be expected, because the environmental conditions are, broadly speaking, changing regularly with latitude and such environmental changes will result in correspondingly adjusted frequencies of the polygenic combination, and hence of the bridling character.

11. Conclusions

Experimental evidence has been advanced for the occurrence of internally balanced combinations of polygenes within chromosomes. Two of these when present in homologous chromosomes can cross over, lose their balance and so release variation, in the sense that the next generation will contain individuals showing an expression of the character outside the range previously encountered. It also appears that such combinations will be developed as a result of natural selection in the wild, since a population heterozygous for a number of these combinations on one or more chromosome pairs would show minimal immediate variation if effective crossing-over were low, and yet by virtue of this rare crossing-over would still include a very few of the extreme types which are necessary for adaptation to environmental changes. Each

polygenic combination is subject to internal balancing by the action of selection in homozygotes and to relational balancing by selection operating on individuals whose homologous chromosomes are carrying unlike combinations.

The foregoing conclusions seem reasonably certain, but beyond seeing that the theory of polygenic combinations throws new light on such diverse questions as inbreeding and outbreeding mechanisms, polymorphism, heterosis and the origin of isolation mechanisms, as indicated above, it will be impossible to make much further progress until more information as to the organization of such combinations is available. We must have information as to the dominance relations of the individual polygenes, on the effect of selection on such dominance relations, of how thoroughly the + and − genes are mixed in the combinations and so on. Of even more importance, perhaps, is a knowledge of how a new combination, formed by crossing-over between two pre-existing balanced types, reacts with its progenitors. Can it differ sufficiently from them to constitute a new combination subject to balancing action, or will it in general be liable to reasonably free recombination with them and so tend to destroy both its own and their individuality? In other words, are the combinations of a population in a perpetual state of flux, with more or less fixed types only maintainable as a result of structural change, or are new combinations in general so unbalanced as rarely to establish themselves? Answers to these questions will do much to advance our knowledge of variation storage and its release in the wild, and the action of both natural and artificial selection.

12. SUMMARY

Polygenic, or "quantitative", variation is present in wild populations and is the raw material of species differentiation. Its properties may be investigated by suitable selection experiments.

The number of hairs on the ventral surface of the fourth and fifth abdominal segments of *Drosophila melanogaster* was chosen as the material for investigation. The *Drosophila* species differ in their hair numbers.

In one cross, $\underline{y} \times f\, B^i B^i$, where selection was exercised from F_2 onwards, the main advance was achieved in the first two selected generations. This was interpreted as being due to recombination of whole chromosomes.

In a second cross, $BB \times +$, following selection from F_2 onwards, an advance with selection was observed for two generations, followed by a

period of stability. This was in turn followed by a second and larger advance with selection, which was interpreted as being due to the action of selection on variation released by recombination of genes in the same chromosome. This conclusion was tested and verified by a repetition of the experiment, by selection within one inbred line and by selection in certain backcross lines. The release of variation by intra-chromosome recombination implies the existence of balanced combinations of polygenes within the chromosomes.

It is shown that such *balanced polygenic combinations* will be developed by the action of natural selection. They effect the best compromise between the advantageous qualities of immediate stability and the ultimate variability necessary for a change in an altering environment.

Polygenic combinations will be subject to an *internal* balancing process in homozygotes and a *relational* balancing process in heterozygotes. The relative efficiency of these processes will affect the breeding mechanism of a population. Heterosis is a result of poor relational balancing and may be the cause of origin of some isolation mechanisms. Polymorphism in nature can be accounted for in terms of the relative efficiency of the internal and relational balances.

It seems likely that a given polygenic combination will have an optimum frequency in a population existing under given environmental conditions. This frequency will change with the environment. If a qualitative gene is completely linked with such a combination it will be expected to show a "cline" of frequencies over large geographical areas, as observed for example in bridled guillemots.

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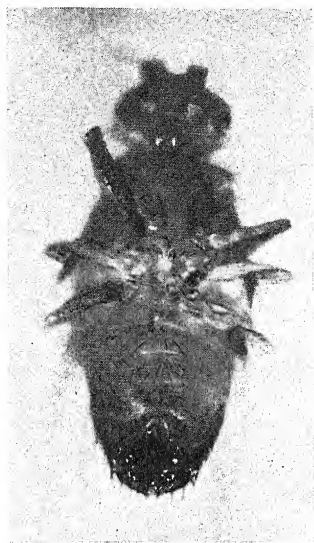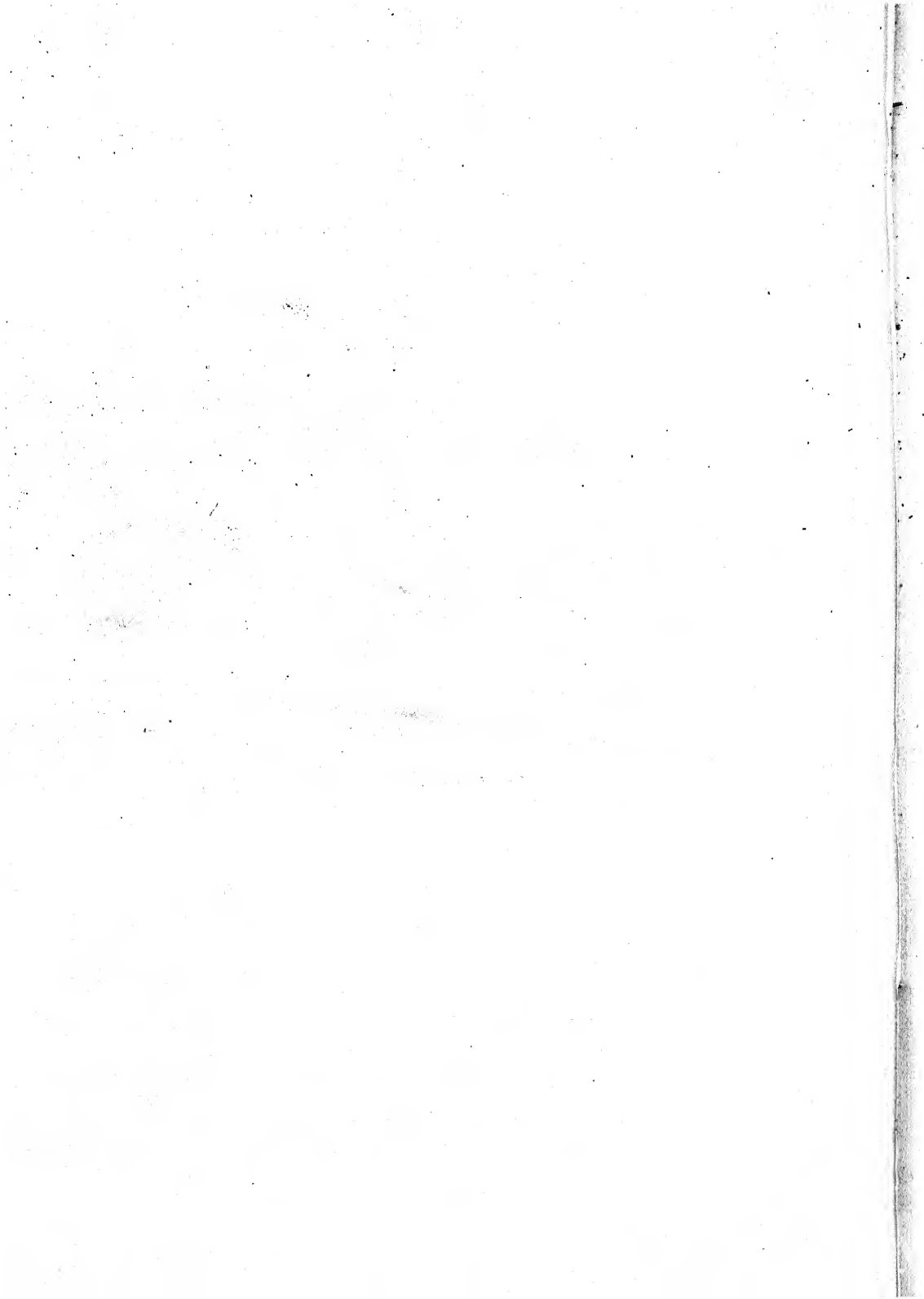
Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

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EXPLANATION OF PLATE 6

Ventral views of various flies to show the abdominal hair clumps (×30 approx.).

Fig. 1. **BB** male from high selection of **BB** × **+**. There are twenty-one hairs on each of the fourth and fifth segments.

Fig. 2. **BB** female from high selection of **BB** × **+**. Twenty-seven and twenty-six hairs.

Fig. 3. Oregon**+** female with twenty-three and twenty-one hairs on the two segments.

Fig. 4. **+** female from the low selection of **BB** × **+**, showing eighteen hairs on each segment.
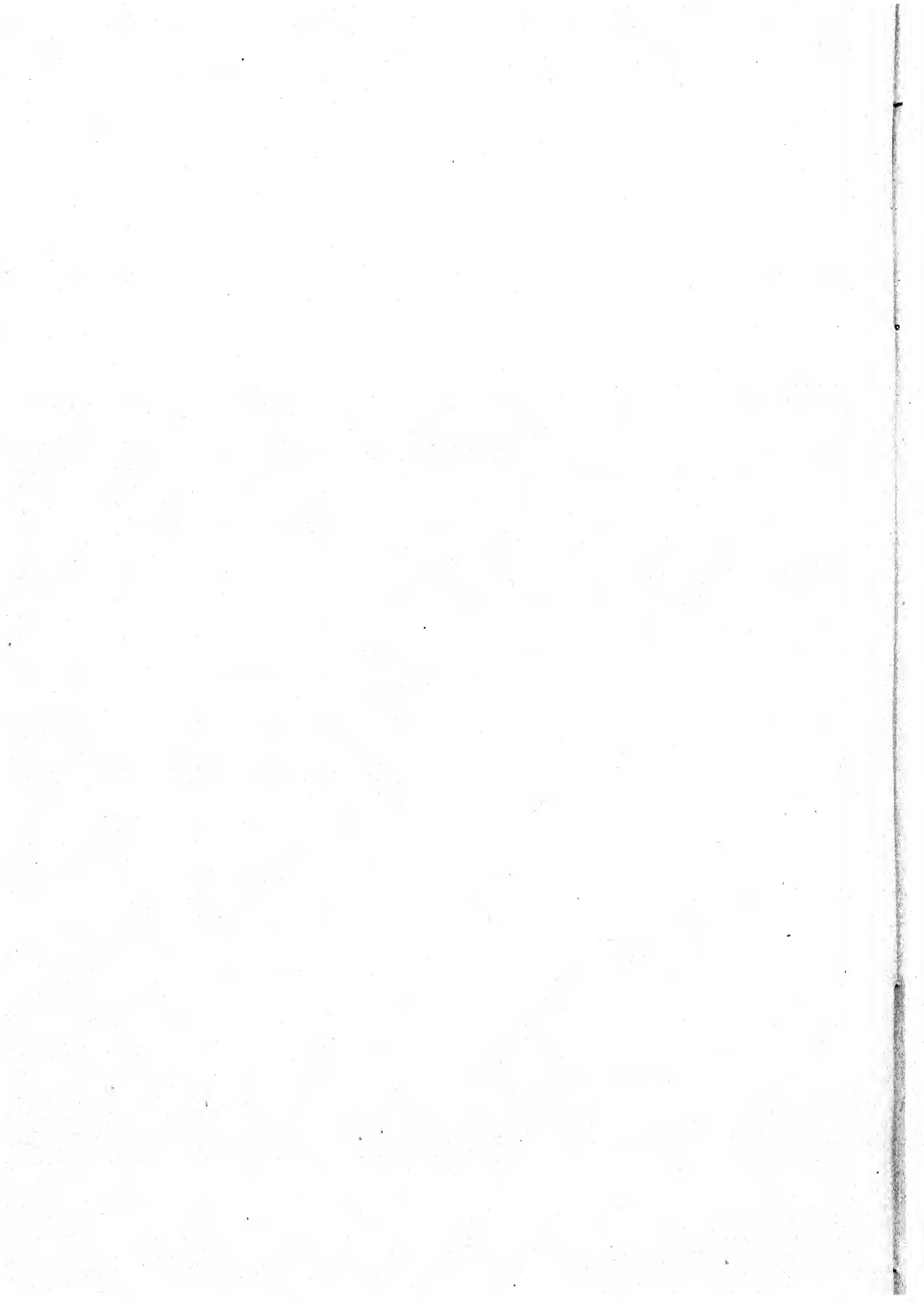
THE INDUCTION OF CHROMOSOME LOSSES IN *DROSOPHILA* SPERM AND THEIR LINEAR DEPENDENCE ON DOSAGES OF IRRADIATION

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(With one Text-figure)

I. THE PROBLEM

THE latest works on the induction of gross chromosome rearrangements by radiation—genetical ones by Muller and co-workers (1938, 1939, 1940) including Belgovsky (1937) on *Drosophila* and cytological ones by Sax (1938, 1939, 1940) and Fabergé (1940) on *Tradescantia* microspores, to quote only those giving the most decisive evidence in this regard—leave no doubt that the process of structural change occurs in two distinct stages. These are (a) primary changes, produced by individual ionizations, and (b) combinations of the primary effects. It is inferred that these two stages consist, respectively, of (a) breakage of the chromonemata at at least two points, and (b) fusion of the broken ends two by two. Presumably this fusion may occur either in the original way (restitution), in which case the effect of the breakage is only temporary, or in a new way, giving a viable or inviable rearrangement. There is evidence that an individual successful ion or pair of ions produced by high-energy radiation—no matter of what wave-length within the range from γ-rays of radium to soft X-rays—is responsible for the production of a break. One of the aims of the present paper is to present further data in support of this proposition.

One of the ways whereby the mechanism of structural change has been investigated, particularly in *Drosophila,* is to study the curve relating the frequency of induced rearrangements to the dosage of irradiation. This curve results from an interaction between several factors: (1) the primary effects of irradiation (presumably the individual breaks), (2) the many possible ways in which the affected chromosomes can join with each other, and (3) effects of differential viability, some of which are dependent, and some not, on the type of rejoining.

In *Drosophila* it was found, for instance, that between 1000 and 4000 or 5000 r. the frequency varies directly as an exponent of the dose, the exponent being about 1·5 (Muller, 1936, 1938, 1939; Belgovsky, 1938;

Bauer *et al.* 1938; Bauer, 1939). The reason of this exponent being 1·5 instead of 2·0, as had earlier been expected on the conception of the mechanism of structural change above outlined, remained obscure until certain valuable contributions to the solution of the problem were presented by Stadler (1936), Haldane (1935—written communication to Muller) and Catcheside (1938). The latter worked out theoretical tables giving the results expected from the interaction of the different effects already described, when certain limiting assumptions were made to simplify the problem. These tables, extended and generalized by Muller and the writer (unpublished, referred to by Muller, 1940), show that, if each break is independently produced by one ionization, the curve relating the dosage with the frequency of obtained rearrangements among total surviving individuals should be an S-shaped one. In its lowest part, that is, nearest the origin, the curve should show the frequency varying with a power of the dosage that approaches 2, and this power should gradually diminish to 1 and even less as the dosage increases. Thus the power, approximately 1·5, experimentally found between 1000 and 4000 or 5000 r., and the highest power, approaching 2, found at very low dosages (Muller, 1940), become explainable.

Catcheside's calculations were based on three assumptions which admittedly do not hold: (1) that only one break per chromosome is produced; (2) that fusion between broken ends is random; (3) that no broken end fails to rejoin either in the original way or with a broken end from a different break. In the above-mentioned calculations by Muller and the author it was shown that the removal of limitations (1) and (2) would not substantially affect the shape of the theoretical curve. As for assumption (3) few pertinent data have hitherto been available. One of the aims of the present investigation concerns itself with this point.

There is genetical and cytological evidence (Muller *et al.* 1937; Muller *et al.* 1938; Belgovsky & Muller, 1938; Belgovsky, 1937; Raffel, 1939) that in *Drosophila*, in contrast with the case of maize (Stadler, 1939; McClintock, 1939), chromosomes without telomeres are generally not capable of functioning indefinitely through a period in which a number of cell divisions occur. And various cases that had been taken to be terminal deficiencies of the *X* were shown actually to be minute intercalary deletions or other minute rearrangements, the very terminal bands being still present. Some further evidence (genetical) of the same kind has been found by the writer (as yet unpublished).

If then chromosomes without a permanent telomere are practically never found in *Drosophila*, this means either that a broken end never

fails to rejoin, either in its original way or with a broken end derived from another end, or else that the two parts produced by a break, one acentric and the other monocentric, must, when not rejoined in one of these ways, become lost by some mechanism or be lethal to the zygote.

The cytological and genetical works of McClintock (1938 *a*, *b*) on maize, and the cytological work of Carlson (1939) on *Cortophaga* are suggestive of one possible mechanism whereby such loss may occur. That is, when the acentric and the monocentric parts of a broken and as yet un-rejoined chromosome undergo mitotic division, the resulting sister chromatids may fuse at their homologous points of breakage and give a V-shaped acentric fragment of double size and a dicentric chromosome, respectively. The acentric fragment may be transmitted for a few cell generations, but sooner or later becomes lost in the cytoplasm. The dicentric chromosome results in the formation of a temporary or permanent anaphase bridge.

McClintock (1938 *b*) had already proved that when there is a ring-shaped dicentric chromosome in maize repeated breakages of the bridges occur at the anaphases of successive mitoses, followed each time by fusion of the non-homologous broken ends, so that the dicentric ring changes in size; it tends ultimately to be lost. Other evidence by the same author showed that small-sized dicentric ring chromosomes are very easily lost through lagging at anaphase. On McClintock's interpretation, if the movement towards opposite poles is initiated by forces acting at the centromere region but continued by other forces exerted on the chromosome when they are some distance away from the equatorial plate, these small dicentric rings may be too small to reach this distance. The final process of loss of an *initially* large dicentric ring may be, then, of the same kind.

Some such process as this has not yet been followed through successive cell generations in other material for non-ring dicentric chromosomes; it is therefore a working hypothesis to assume that, in its main lines, it may take place in *Drosophila* both with ring and non-ring chromosomes. In the non-ring, there would be this difference: the fusion could not occur until later, after chromosome division, and would then join homologous broken ends of sister chromatid fragments. Further, the possibility must be considered that in *Drosophila* breakage of a dicentric chromosome does not occur, but that, as in the case of McClintock's small rings, the dicentric fails to be included in either daughter nucleus.

Now, if the ends derived from breaks produced by irradiation in the chromosomes of *Drosophila* sperm sometimes fail to rejoin in time, a

fusion of sister chromatids at the point of breakage may occur at the first splitting following irradiation,[1] and either cause immediate loss or start the process of repeated breakage at anaphase. In either case, on this assumption, the formation of a sister-chromatid dicentric is the first step in the process of loss. As these losses should be produced by single breaks, in contrast with all gross rearrangements, which are supposed to require two or more breaks, the frequency-dosage relationship of the former should be different from that of the latter. If the "primary changes" already mentioned are really the breakages, then these losses, being due to single breakage, should vary in frequency no more rapidly than the first power of the dose.

The finding of such a result—contrasting so much with that found for gross rearrangements—would thus help to fill the gaps in the structure of evidence in support of the "breakage first" theory of chromosome rearrangements. Some evidence to this effect has recently been presented by Muller (1939, 1940), but, as he stated at the time, that work was only preliminary and a more systematic and extensive attack (that to be reported herein) was already in progress under his direction.

The group of problems which it was proposed to investigate in our present work was accordingly the following: (*a*) whether or not irradiation of *Drosophila* sperm results in chromosome losses which under suitable conditions are non-lethal to the zygote and which may be attributed to broken ends that fail to rejoin either in the original way or with ends from other breaks; (*b*) if the answer be affirmative, what is the frequency-dosage relationship for these losses and what light may it throw on the validity of our interpretation that the losses are due to single breaks; and (*c*) what light may it throw on the interpretation of the shape of the frequency-dosage curve for gross rearrangements.

On the technical side the problem was therefore that of detecting losses of whole chromosomes, such as might be produced when a dicentric chromosome arises by fusion of the broken ends of sister chromatids. Here a special method, involving the use of the *sc*. Y^L chromosome (vide infra), was devised by Muller; this represented a technical advance over his earlier described method (1939, 1940), in which a dominant variegated allele of brown had been used for disclosing the presence of an ordinary *Y*-chromosome.

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[1] The assumption (Patterson, 1933; Mather, 1937), made to explain "fractional" rearrangements, that the chromosomes are in part or all split in the *Drosophila* sperm is, as Muller (1939, 1940) points out, no longer necessary, since evidence has been obtained that rearrangements are not completed until after fertilization.

II. Technique adopted for detection of losses of X and Y

Among the possible methods for genetically detecting the loss of a chromosome, the first that comes to the mind, so far as the X and Y are concerned, is the utilization of the mechanism of sex determination. Any method of this kind, however, presents several practical difficulties, one of which is the great number of cultures required to obtain statistically sound results, and another the fact that effects simulating losses, and distinguishable from them only with difficulty, are produced in several ways.

It was therefore thought that some way of distinguishing attached-X females carrying a Y from those not carrying it would be more promising. If a potential loss of the X-chromosome can be induced in an X-bearing sperm and this happens to fertilize an $X.X$ egg,[1] the resulting zygote will be a perfectly viable $X.X/O$ female, in general phenotypically indistinguishable from an $X.X/Y$ one. The same result will be obtained if loss of the Y-chromosome is induced in a Y-bearing sperm. Hence the possibility of detecting losses of X or Y chromosomes induced in the sperm depends upon suitable methods of distinguishing, by inspection, $X.X/O$ from ordinary $X.X/Y$ females.

An excellent opportunity in this direction was provided by the chromosome designated as $sc.Y^L$ (Crew & Lamy, 1940). This chromosome had originally been described as rod-shaped, but co-operative investigations (Pontecorvo, 1940 a) showed it to be **V**-shaped: one of the two arms is the Y^L and the other is even longer than the Y^L. This latter (longer) arm is provisionally described as composed, starting from the centromere, of the main part of the proximal region of the Y^S, including its main block and probably bb^+ but excluding some or all of the more distally located fertility genes (Neuhaus, 1939), then of a large part of the proximal heterochromatic region of the X (including block A and bb^+), and finally of the left tip of a sc^{S1} chromosome, extending from its original left break to the end, and thus including the genes for sc^{S1}, ac^+ and y^+. The presence of this distal X-chromosome component, carrying y^+ and the other markers mentioned, makes the $sc.Y^L$ valuable for our purpose. In fact, a cross of males with the $sc.Y^L$ to $y.=$ females will give daughters all of which ordinarily have normal grey body colour, the yellow of their attached-X's being "covered" by its wild-type allelomorph carried in the

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[1] In the notation used above, and throughout this paper, a full stop indicates attachment of the two parts shown on either side of the stop. When the two parts are alike (homozygous) the sign $=$ will be used after the stop; thus $y\,vf.=$ means attached-X's homozygous for $y\,v\,f$ (see Muller, 1934).

sc component of the paternal *sc*. Y^L. Except for mutations or deletions of y^+, only females of type $X.X/O$, originating when the paternal *sc*. Y^L or X is "lost", will show the yellow body colour. The P_1 males used must of course contain, for fertility, a Y^S (short arm of the Y) also; it is convenient to have this attached to their X-chromosome, to the right of the centromere; thus their composition is $X.Y^S/sc.Y^L$.

The preliminary tests by Muller (*op. cit.*)—with the method based on the distinction of $X.X/O$ from $X.X/Y$ females by means of the effect of the extra Y in suppressing the eye-colour variegation caused by a dominant allele of *bw*—had shown that the frequency of induced non-lethal losses of X and Y was rather low (of the order of 2 % with 4000 r. in his material). It was therefore necessary to have a stock with a very low *spontaneous* frequency both of those events which lead to a loss and of those which give the same phenotypical effects as a loss, namely: (*a*) non-disjunction in the males; (*b*) mutation to yellow in the *sc* component of the *sc*. Y^L; (*c*) release into circulation in the stock of an extra Y, or of parts of Y, not having the "marked" *sc* component.

The stock first tested had its males of composition $y\,X.Y^S/sc.Y^L$ and females $y\,w\,f.=/sc.Y^L$. In mass cultures of this stock some phenotypically y males, $y\,w\,f$ females and wild-type females appeared. The phenotypically y males and $y\,w\,f$ females proved in different cases to be of non-disjunctional origin, in which case they carried no Y, or to carry a whole Y, a Y^S or a Y^L without the *sc* component.

The presence of a whole Y in circulation in the stock could easily be explained by a crossing-over in the male between the *sc*. Y^L and the $X.Y^S$, giving an X with a *sc* duplication, on the one hand, and a whole Y on the other hand. The presence of a Y^L deprived of the y^+ marker could also be explained easily, as a spontaneous "mutation" to y (or better, a minute rearrangement) in the *sc* component of the *sc*. Y^L. The rate of this spontaneous "mutation" has been measured by Belgovsky (1938, 1939) and by Muller & Makki (1938; Muller, 1939, 1940) as some 1 in 10,000 in an inverted X-chromosome (sc^8) in which, as in the *sc*. Y^L, the y^+ locus is very close to a portion of heterochromatic region that had originally been proximal in location. The manner of origination of the Y^S, however, was not so obvious until the following fact was observed by Miss Lamy and analysed by the writer (Lamy & Pontecorvo, 1940): a part of the phenotypically wild-type females proved to be of composition $y^+.=/sc.Y^L$, namely, newly originated $X.X$ females with their attached-X's of the same type as the paternal X. A fact of the same kind had already been briefly reported by Neuhaus (1936). The origination of these $X.X$ females

can be explained by a crossing-over between different arms of sister chromatids in the paternal $X.Y^S$ chromosome; its complementary product must be a free double Y^S ($Y^S.=$). This would account also for the presence of the Y^S found genetically to exist in the stock.

Incidentally, this mode of new origination of attached-X's, which has been successively detected in several different $X.Y^S$ and $X.Y^L$ stocks, can be of great practical use for the building of any kind of $X.X$ stock. Moreover, if this may be a method of spontaneous origination of attached-X's, then these chromosomes are not necessarily iso-chromosomes (Darlington, 1939). Perhaps investigations in this direction would afford a test for deciding between such an origin for these cases and that proposed by Darlington (misdivision of the centromere).

The following measurements of the *primary* frequency with which the yellow exceptional females arise (some 1·5 %) showed that it was far too high for making the above stock suitable for our purpose:

Table 1. *Primary exceptional flies in the stock of composition*
$y\,X.Y^S/sc.\,Y^L\,\male$ *and* $y\,w\,f.=/sc.\,Y^L\,\female$

Ordinary females phenotype: wf	Exceptions (phenotypes)		
	$+\,\female\female$	$y\,w\,f\,\female\female$	$y\,\male\male$
1255	1	18	2

A new stock, built up with the same $y\,w\,f.=/sc.\,Y^L$ females but using males with an $X.Y^S$ chromosome of different origin, marked by the recessive forked, gave the following satisfactory results (Table 2):

Table 2. *Primary exceptional flies in the stock of composition*
$f\,X.Y^S/sc.\,Y^L\,\male$ *and* $y\,w\,f.=/sc.\,Y^L\,\female$

	No. of flies
Ordinary females	6419
Exceptions, classified phenotypically:	
Females $y\,w\,f$	3
Females $\quad f$	2
Males $\quad w\,f$	1 (detachment of the $X.X$)

The different behaviour of the two stocks as regards non-disjunction is probably not caused by the $sc.\,Y^L$ but by some peculiarity of the $X.Y^S$, resident in its proximal regions. As the above results show, this second stock was found to be suitable for the proposed experiment.

To avoid the dissemination of whole Y-chromosomes or of either of the arms of the Y originating in the manner already explained, the stock was maintained by the inbreeding of individual pairs of brothers and sisters, with continual elimination of families in which an undue proportion of exceptions appeared.

III. Experimental set-up, and F_1 results

X-rayed males of the stock previously described—$f X . Y^S / sc . Y^L$—were crossed to $y\, w\, f. = / sc . Y^L$ females, and the exceptional (yellow) females appearing in F_1 were recorded.

The following technique was adopted: the males collected from inbred pair matings, when 1–3 days old, were thoroughly mixed and divided into three groups. The males of one group, used for control, were mated individually with two virgin females. The other males were X-rayed at room temperature (about 16° C.) with a broad-focus water-cooled Coolidge tube (tungsten target) operated at 8 mA. and 70 kV.; a 0·5 mm. Al. filter was used. The intensity on the flies was about 350 r. per min. Two doses were given, namely, 1000 and 4000 r. The relative values of the two doses (1:4) in this experiment have considerable accuracy, owing to the technique described below. The same cannot be said of the absolute values.

For the high dose the males were exposed to the irradiation for about 10 min. For the low dose, the males were divided into four equal groups and each group irradiated together with the males of the high dose, but for a length of time such as to give only one-quarter of the high dose. That is, after each quarter (1000 r.) of the high-dose treatment had been given, one of the four groups of low-dose males was removed and another one substituted. This method, first adopted by Offermann in 1934 (unpublished), has the advantage of equalizing to a considerable extent variations in the conditions of treatment that might otherwise affect the high- and low-dose groups differentially.

Immediately after treatment, the males were mated individually in vials with virgin females, six females for each male of the high-dose group and three for each male of the low-dose group. After $4\frac{1}{2}$ days the males were discarded and the females of each vial transferred to fresh vials. The cultures were maintained at a constant temperature of $24 \pm 0\cdot5°$ C. and were examined when almost all the pupae had hatched; at this time, practically all of the imago progeny were still alive.

The method of individual matings was adopted so as to make possible the detection of male parents carrying an unmarked Y^S or other part of a Y, instead of, or in addition to, a $sc . Y^L$. For the presence of such parts would have given results in the next generation simulating loss. No cases of this kind occurred, however, in the 1020 individual matings of the above experiment. Males with such aberrations are to be expected with an incidence of some 3 in 10,000, provided the stock is always multiplied,

as it actually was, by inbred pair matings, so as to exclude secondary occurrences.

The count of F_1 females from this experiment is shown in Table 3. Among the eighty-six exceptional flies found in the F_1 from the males

Table 3. *F_1 females from frequency-dosage experiment*

Dose in r.	Ordinary ♀♀ (phenotype $w f$)	Exceptional ♀♀ (phenotype $y w f$)	% exceptional
4000	4,987	$84 + 2(\frac{1}{2})$*	1.70 ± 0.18
1000	10,427	56	0.54 ± 0.07
Control	7,071	5	0.07 ± 0.03
Total	22,485	146	—

* Two cases of "fractionals".

treated with the high dosage, two were mosaics of the "fractional" type (Muller, 1927). As the results obtained in genetic analyses of these and many other similar "fractionals" found in a parallel series of experiments throw an interesting light on the mechanism of induction of structural changes, a detailed discussion of the problem will be given elsewhere. It is sufficient here to point out that these fractionals are not counted as exceptional units, but as halves.

IV. Tests of the exceptional flies

The exceptional flies are detected by their yellow body colour; this character, in the regular flies, is "covered" by the wild-type allele carried in the *sc* component of the *sc*.Y^L.

In addition to the complete loss of the $X.Y^S$ or *sc*.Y^L, several other ways are conceivable whereby the same phenotypical effect can be produced. These are:

(*a*) presence of a normal Y, or of one of its two arms, carrying no marker;

(*b*) minute deletion or other minute rearrangement in the *sc* arm of the *sc*.Y^L chromosome, producing a loss or change to yellow of its wild-type allelomorph; actual gene mutation of the y^+ gene in this chromosome to y —a much rarer phenomenon—may also be included here;

(*c*) large deletion of the $f X.Y^S$ chromosome, with left break to the left of y^+ and right break between w^+ and the centromere;

(*d*) large deletion of the $f X.Y^S$ chromosome, producing a ring-shaped chromosome with left break between w^+ and the centromere and right break anywhere in the Y^S arm, but proximal in respect of at least one of its fertility genes, or producing a sterility position effect in it;

(*e*) deletion of the *sc.* Y^L chromosome, producing a ring-shaped chromosome with one break in the *sc* arm, proximal in respect of y^+ or producing "mutation" of it to yellow, and the other break anywhere in the Y^L arm but proximal in respect of at least one of its fertility genes or producing a sterility "mutation" in it;

(*f*) "mutation" (minute rearrangement of some kind or actual gene mutation) to *y* in the *sc* arm of the *sc.* Y^L and simultaneous sterility "mutation" in the Y^L;

(*g*) translocation of the *f X.* Y^S or *sc.* Y^L to the fourth chromosome with acentric-dicentric reunion of the broken ends and loss of both chromosomes;

(*h*) complex rearrangements resulting from combinations of the already described simple ones.

Cases (*a*) are consequences of crossing-over in the male and are therefore independent of the X-ray treatment. The control series were carried out largely for the purpose of checking this occurrence.

The occurrence of the other types had to be ascertained by tests carried out on the exceptional flies themselves. The following technique was adopted:

In *cases* (*b*) *and* (*c*) the Y^L arm of the *sc.* Y^L chromosome, or the Y^S arm of the *f X.* Y^S chromosome, respectively, remains unaffected. An $X . X$ exceptional female carrying either will transmit it to her sons. If these sons carry, attached to their *X*-chromosome of paternal origin, the complementary arm of the *Y*, they will have the whole set of fertility genes and will be fertile (Stern, 1929; Neuhaus, 1939). Therefore by mating each exceptional female to *X.* Y^S and *X.* Y^L males successively and testing the sons of each for fertility, it can be ascertained whether or not she carried one of the arms of the *Y*, and, in case she did, to identify it (Muller, 1939, 1940).

In *cases* (*d*) *and* (*e*) the fertility genes—several of which have been shown (Neuhaus, 1939) to be located in the distal portions of each arm of the *Y*—are affected and therefore none of the preceding tests is suitable. However, the presence of bb^+ in the proximal regions both of the *X.* Y^S and of the *sc.* Y^L suggested a test based on the detection of this gene. Males of a *bb*-deficient stock, *y sc⁴ L In-S sc^{S1} R*, obtained by Muller by crossing-over between the *sc⁴* and the *sc^{S1}* inverted chromosomes, are viable only if the deficiency of bb^+ in the *X* is "covered" by its presence in the *Y* or elsewhere. From the cross of a male of such type with an exceptional nullo-*Y* attached-*X* female, no sons are produced except for a few *fertile* males resulting from non-disjunction of the *X* and *Y* in the

father. If, however, the exceptional female tested carries a Y^S, a deleted $f\,X\,.\,Y^S$ or a deleted $sc\,.\,Y^L$ in which there is an unaffected bb^+ gene, a greater number of males will be produced, and these will be *sterile*. In an $X\,.\,Y^S$ chromosome there are two loci containing bb^+, both proximally placed. In a $sc\,.\,Y^L$ chromosome at least one locus with bb^+ is surely present in the proximal part of the sc-containing arm, while no such locus, or one with only a very weak bb allele, is present in its Y^L arm, as results obtained in our own investigations have shown. If a ring is formed in the $X\,.\,Y^S$ or in the $sc\,.\,Y^L$ as a result of two breaks occurring on opposite sides of the centromere, followed by joining of the broken ends, there is a good chance for one of the breaks to have been distal in respect of a locus containing bb^+. This chance must be very high for rings formed from the $X\,.\,Y^S$, because of the two bb^+-containing loci originally present, one in each arm, in proximal positions. When the ring is formed in the $sc\,.\,Y^L$, having a bb^+-containing locus or loci in only one of its arms, the chance of this locus being included is lower.

Case (f)—a mutation in one of the fertility genes carried in the long arm of the $sc\,.\,Y^L$ chromosome coinciding with a y "mutation" in its sc component is also distinguishable from complete loss by the test for the presence of bb^+.

Case (g)—an aneucentric (acentric-dicentric) translocation of the $X\,.\,Y^S$ or the $sc\,.\,Y^L$ with the fourth chromosome—would produce, through elimination of both the chromosomes concerned, an exceptional (yellow) fly which would also be haplo-IV and easily recognizable as such. No exception of this kind occurred in the experiment here reported.

All the induced rearrangements described require one (case b) or two ionizations (cases c, d, e, f, g). The occurrence of cases due to three ionizations must certainly be less frequent. Most of them, however, could be distinguished from losses—with the same limitations as for the rings—by means of the test for bb^+.

All the types from (a) to (f), and in addition some of the more complex types of exceptional flies carrying different parts of the $sc\,.\,Y^L$ or $X\,.\,Y^S$ chromosomes, actually occurred as a consequence of X-ray treatment. Fig. 1 shows, for instance, the metaphase plate of a ring resulting from one break in each arm of the $sc\,.\,Y^L$.

Summing up the method: three tests had to be carried out with any given exceptional (yellow) female before it could be ascertained to which type she belonged. These were:

(1) Test for the presence of the whole set of fertility genes carried by the Y^L.

(2) Test for the presence of the whole set of fertility genes carried by the Y^S.

(3) Test for the presence of bb^+.

Exceptional females giving negative results with all the three tests can be considered as being nullo-Y females in the great majority of cases. The same results are, to be sure, given when a ring chromosome not including bb^+ is carried by the female as when a female is nullo-Y. But such cases are probably a small fraction of the total, judging by the small number of rings that do contain bb^+. For the tests of earlier workers on deleted X-chromosomes (Muller & Painter, 1929; Muller & Gershenson, unpublished) have shown that in a large proportion of deleted X's

a

b

Fig. 1. A ring-shaped chromosome resulting from one break in each arm of the *sc*. Y^L. Mitotic metaphase plate of a female carrying attached-X's, a normal Y and the ring. *a*, camera lucida drawing (× 3500) of same plate as in microphotograph *b* (× 800).

having one break in the heterochromatic region the locus of bb has not been removed.

Practically, the three tests were carried out as follows:

The exceptional flies, when collected, were already inseminated by their $f X . Y^S/sc . Y^L$ brothers. A period of 4–5 days laying (each female being kept individually) was sufficient to give rise to enough offspring for the first test. The sons coming from any given culture of this "brood" were mated in mass (5–10 males with 5–10 females) to their sisters. In case they proved to be fertile, the test was verified by mating 5–10 such males *individually*, each male with 2–3 virgin females, so as to exclude cases of fertility of males due to the occasional origination of males by non-disjunction. A positive result from this test (i.e. fertility of many of the sons) was considered as a proof that the exceptional fly had carried the whole set of fertility genes of the Y^L.

The same exceptional female, discharged of a part of the sperm received in the first type of mating, was then mated to males of the composition $X.Y^L/Y^S$. The two progenies were easily distinguishable because the daughters from the first type of mating, carrying the paternal $sc.Y^L$, were phenotypically $y^+ wf$ and the sons f, while the daughters of the second type of mating were $y wf$ and the sons wild-type. The sons derived from this second type of mating were tested for fertility in the manner already described for the ones from the first mating. A positive result of this test was considered as proof that the exceptional fly had carried the whole set of fertility genes of the Y^S.

After the second type of mating, the fly was transferred to fresh food for 4–5 days and then transferred again and mated, this time with $y sc^4 L In-S sc^{S1} R$ (bb-deficient) males, marked in one of their second chromosomes by Cy and L^4. If no yellow scute sons appeared in the progeny, but many females, about half of them $Cy L^4$, the result of the test for the presence of bb^+ was considered as negative. Usually, however, even when a deleted chromosome carrying bb^+ was not present in the exceptional fly, a few sons appeared in consequence of the comparatively high frequency of primary non-disjunction (at least 5 %) occurring in the males of the $y sc^4 L In-S sc^{S1} R$ stock. In this case these sons were tested individually for fertility (presence of a whole Y) to confirm their non-disjunctional origin. When, on the other hand, many males appeared in the progeny and on being tested individually proved in great majority to be sterile, the result of the test was considered positive, and the conclusion was drawn that the exceptional fly carried a deleted, probably ring-shaped, $sc.Y^L$ or $X.Y^S$.

The impossibility of carrying out the above triple test, with its various ramifications, on all the exceptional flies is obvious. Each fly, already some days old when collected, must give progeny with three different types of males. Often the fly died before giving the whole series of progeny. The tests which were carried through were, however, sufficiently numerous to show the trend of the results, and to allow certain definite conclusions to be drawn. The results of these tests are summarized in Table 4.

In this table only those flies are recorded which gave at least the first two kinds of progeny, whereby the presence of the whole set of fertility genes of each arm of the Y is detected. The third kind of progeny—from bb-deficient males—was obtained with about half of the flies which had already passed through the other two tests.

The results indicate that the majority of the exceptional females—

roughly two-thirds in the treated series—are nullo-Y. Furthermore, the proportion of exceptions which are nullo-Y is, in first approximation, of the same order for the two doses (although with larger numbers we should expect the excess of various kinds of incomplete chromosomes formed at the higher dose, as compared with the lower, to become significant).

Table 4. *Data on percentage of exceptional F_1 ♀♀ which constitute cases of complete loss of $X . Y^S$ or of $sc . Y^L$*

	Treatment		
	4000 r.	1000 r.	Control
A. Exceptional ♀♀ tested for the fertility genes in both arms of the Y	43	35	4
(*a*) Found to carry the whole set of fertility genes of Y^L only	9	3	2
(*b*) Found to carry the whole set of fertility genes of Y^S only	3	5	0
(*c*) Found to carry both the sets of fertility genes of Y^L and Y^S	0	0	0
B. Total in category A found to carry the whole sets of fertility genes of one or both arms of Y	12	8	2
C. Remainder, carrying neither whole Y^L nor whole Y^S	31	27	2
D. Exceptional flies in category C tested for presence of bb^+	19	12	2
E. Flies of category D found to carry a deleted $X . Y^S$ or $sc . Y^L$ with bb^+	2	1	0
F. Remainder of category D, not carrying bb^+	17	11	2
G. Proportion of exceptional ♀♀ carrying no whole arm of Y (C/A)	$\frac{31}{43}=72\pm7\%$	$\frac{27}{35}=77\pm7\%$	$\frac{2}{4}=50\pm25\%$
H. Proportion of preceding carrying no deletion with bb^+ locus (F/D)	$\frac{17}{19}=89\pm6\%$	$\frac{11}{12}=92\pm8\%$	$\frac{2}{2}=100\pm7\%$
I. Percentage of exceptional ♀♀ representing complete loss of $X . Y^S$ or of $sc . Y^L$ (G × H)	$64\pm7\%$	$71\pm9\%$	$50\pm25\%$

The conclusion can be drawn that complete losses of the X- and Y-chromosomes are in fact induced by irradiation of the mature sperm. Moreover, those losses here demonstrated are not lethal to the zygote. Whether the latter characteristic holds for *all* the induced losses of X- and Y-chromosomes or only for a part of them—those which can be detected by the present method—will be discussed later.

V. The frequency-dosage relationship

The data summarized in Table 3 give a frequency of $1\cdot70\pm0\cdot18\%$ exceptions at the higher dosage, of $0\cdot54\pm0\cdot07$ at the lower dosage, and of $0\cdot07\pm0\cdot03$ in the controls, the dosage ratios among the three series being $4:1:0$. The tests of the exceptional flies (Table 4) have shown that the proportion of losses of the whole $X . Y^S$ or $sc . Y^L$ chromosome among

them is 64 ± 7, 71 ± 9 and $50 \pm 25 \%$, respectively, for the three series. We have then the data for determining three points of the curve relating the dosage of irradiation with the frequency of losses, namely for those points corresponding to the abscissae 0, 1000 and 4000 r. This makes it possible to test whether or not, at a first approximation, this relationship can be represented by a linear equation (straight line).

The experimental evidence in *Drosophila* shows that the frequencies both of induced "point" mutations and of minute rearrangements increase, within the dosages here used, approximately in linear manner, that is, as the first power of the dosage. The frequency of gross rearrangements, on the other hand, increases, within the same range, as an exponent of the dosage higher than 1 and lower than 2: approximately as the 1·5 power (see review of the matter by Timoféeff-Ressovsky for "point" mutations and by Muller for rearrangements at the Seventh International Congress of Genetics, 1939).

The present data on the frequency of exceptional flies at the three above-mentioned doses can be interpolated by a linear equation, as shown in Table 5. The agreement between the data found and those

Table 5

Dose	Exceptional flies found %	Cases of loss of whole $X . Y^S$ or $sc . Y^L$ among exceptional flies %	Frequency of losses	
			Found (B × C) %	Expected* %
A	B	C	D	E
0	$0·07 \pm 0·03$	50 ± 25	$0·035 \pm 0·03$	0·077
1000 r.	$0·54 \pm 0·07$	71 ± 9	$0·38 \pm 0·07$	0·334
4000 r.	$1·70 \pm 0·18$	64 ± 7	$1·09 \pm 0·16$	1·105

* Interpolating equation $Y_d = \bar{y} + 0·257 (d - \bar{d})$, where $Y_d =$ percentage frequency at dosage d (in 1000 r.).

expected is very good, being $\chi^2 = 0·04$, a value which, for one degree of freedom, is exceeded by chance in more than 80 % of cases. The slight departure from linearity is, in any case, in the direction of an exponent lower than 1. And the departure in this direction would have been even greater if there had been a more distinct excess of incomplete chromosomes among the exceptions at the higher dose, as a greater body of data would have been expected to show.

At the same time, the possibility had to be tested that a curve in which the frequency of losses varied as the 1·5 power of the dose, like the curve for gross rearrangements, would also fit the data sufficiently well. The expected value for 1000 r. may be calculated on this assumption from the 4000 r. value, as the latter is affected by a proportionately lesser

error. Allowing for the controls, this expected value for 1000 r. is 0.13 ± 0.02, as compared with the obtained value of 0.38 ± 0.07. The difference, 0.22 ± 0.07, is some three times its standard error. Thus the frequency-dosage relationship is suitably expressed by a linear equation and cannot be expressed by an equation having the exponent 1·5. It may be concluded that, within the range of X-ray dosages used in this experiment, the frequency of losses appears to be, in first approximation, a linear function of the dosage of irradiation, and that it does not vary as rapidly with dosage as the frequency of gross structural changes does.

It must be observed that the way in which the frequency of losses has been expressed here is an empirical one and does not represent the actual frequency *per treated sperm*. In fact, when a non-lethal loss is induced in an X-bearing sperm fertilizing an $X.X$ egg, a viable exception of composition $X.X/O$ results from a zygote which otherwise would have been an inviable triplo-X. The frequency of losses per treated sperm is therefore only half that given in the above table, namely 0·17 % with 1000 r. and 0·55 % with 4000 r.

VI. DISCUSSION

The results reported above show (*a*) that, in agreement with Muller's preliminary evidence, losses of the whole X- or Y-chromosome are actually produced by irradiation of the mature sperm and that they are, in part at least, non-lethal to the zygote; (*b*) that between 0 and 4000 r. their frequency increases—at a first approximation—as the first power of the dosage and certainly less rapidly than the *ca.* 1·5 power characteristic of gross rearrangements involving two or more breaks.

As for the frequency-dosage relationship, although there is no significant departure from linearity, the evidence indicates that, if any exists, it is in the direction of an exponent even lower than 1. It is to be expected that a refinement of the experiment would show this slight departure from linearity to be real, for such a departure would arise from (*a*) the fact that the basically linear curve would tend to "saturation", i.e. at higher dosages the coincidence of two or more breaks, each leading to loss, becomes greater; and more especially from (*b*) the fact that, with an increased number of breaks per nucleus, greater opportunity would be given for a break to take part in a lethal or non-lethal gross rearrangement (especially a translocation) instead of in a loss.

A most interesting preliminary report by Bauer (1939 *b*) has just appeared in which the induction of losses of the Xc^2 ring chromosome was investigated by measuring the amount of change caused in the sex-ratio

when irradiated males bearing the ring were crossed to females with normal X's. The results, expressed as the ratio of males minus females to males (all corrected for controls), when plotted against dosage gave "an oversaturated one-hit curve, which results from the difference between a one-hit and a two-hit curve. The former suggests the proportionality to the dosage of the production of dicentric double rings, the latter corresponds to the two-hit curve of the lethal (aneucentric) Y-translocations."

In an experiment by the writer (1940b), in which the frequency of losses of X-chromosomes of different lengths and structures were investigated by the earlier method of Muller (1939, 1940), involving the difference in expression of dominant alleles of brown shown by $X.X/Y$ and $X.X/O$ females, viable losses of the Xc^2 chromosome proved to be induced, at 4000 r., with a frequency[1] significantly greater than $2\cdot8 \pm 0\cdot5\%$ per treated X-bearing sperm. This figure represents only real losses; therefore it is not masked, as Bauer's is, by other effects, such as an excess of dominant lethal gene and chromosome mutations arising in the Xc^2 as compared with the Y, and "conversion" of females into males by deletion of the X.

Now the graph published by Bauer shows a deficit of some 40% in the number of females as compared with that of males for the same dose, 4000 r., and recent tests by Muller and the writer (unpublished) confirm this result. On the other hand, the frequency of viable losses of Xc^2, found in our experiment cited above, when calculated in a way comparable to Bauer's, turns out to be about 5%. Thus the remainder of the deficit of females, 35 out of the 40%, must be ascribed almost entirely (that is, except for the "conversions" due to deletion above mentioned) to an excess of dominant lethals arising in the Xc^2-bearing sperm, associated either with losses of the X or with gene mutations or minute or gross structural changes of it, as compared with those arising in the Y-bearing sperm. When a non-ring X-chromosome is used, some 10% of eucentric translocations, mostly two-break ones, involving the X are obtained with 4000 r. Both these and the complementary aneucentric translocations are lethal in the case of an Xc^2 chromosome, but in the

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[1] The frequency of losses with the Xc^2 chromosome proved to be more than twice as high as that with a rod-shaped X. This is in agreement with expectancy on the basis of the proposed mechanism for the origin of losses of the rod-shaped X—breakage followed by fusion of sister chromatid fragments, producing a dicentric—because in a ring chromosome two, out of the three possible ways in which the broken ends of chromatid fragments may rejoin two by two, lead to formation of a dicentric, while in a rod-shaped chromosome only one out of these three possible ways gives this result. The matter is explained in detail in the other paper (being prepared for the Press).

case of the *Y* (which is involved in translocations with roughly the same frequency as the *X*) only the latter would be lethal, leaving a difference of 10 %. A further group of some 10 % can be ascribed to dominant lethals (gene mutations and minute and gross rearrangements other than aneucentric translocations) arising in the *X*, as was shown by Muller (1928) and confirmed by data of several authors (Barth, 1929; Gowen & Gay, 1933; and preliminary results recently obtained by Muller and the writer). The remainder of the deficit—apparently some 15 % at least—is evidently produced by lethal losses of the Xc^2 or, to be more exact, by the difference between lethal losses of the Xc^2 and lethal losses of the *Y*, should the latter losses exist at all.

From the above rough estimates the lethal and non-lethal losses of the Xc^2 appear to occur in a ratio of about 3 : 1, and losses of both kinds together to account for at least one-half of the change in the sex ratio observed by Bauer with the 4000 r. dosage. The other half, then, is constituted of a mixture of other "one hit" effects and of "two or more hit" effects. The proportions of losses, of other "one hit" and of "two or more hit" effects change with the dosage, the frequencies of the former two being preponderant at low doses and increasing linearly while that of the latter increases as a power of the dosage that is at first 2 and gradually diminishes to 1·5 and less as the dosage rises. It seems therefore that an exact allowance for all these different effects is necessary before conclusions concerning the dosage-frequency relation for losses can be drawn from Bauer's results. However, a combined use of Bauer's method and that adopted here may afford a more nearly adequate means of approach than either of the two alone. For with the former, all losses—lethal and non-lethal—are scored, but there are other overlapping effects, difficult to allow for, while with the latter there is not such an interference of effects, but there is the drawback that only non-lethal losses are detected.

The linear proportionality to dosage found in the present work for non-lethal losses was expected on the basis of the loss mechanism postulated; in fact, if losses are produced by single breaks, and single breaks by single successful ionizations, the frequency-dosage relationship expected is, at a first approximation, that of a one-hit curve, i.e. a straight line. This result argues strongly, from a new angle, for the "breakage first" theory of structural changes, according to which a power of the dosage higher than the first is expected for expressing the frequency of gross rearrangements (two or more breaks) and a power equal to or lower than the first for single-break events, as losses are supposed to be. The possibility cannot yet be excluded, however, that the mechanism of losses may

be entirely different from that proposed in this paper, as for instance by some action of the radiation on the centromere or on achromatic constituents of the mitotic apparatus. Parallel experiments to clarify this point are in progress.

The fact that lethal and non-lethal losses together proved to constitute a considerable portion of structural changes shows that also assumption 3 (p. 198)—made when calculating the frequency-dosage curve for gross rearrangements—does not hold (provided the mechanism of losses here advocated is correct). However, as the frequency of losses proved to be roughly a linear function of the dosage, allowance for it would not substantially alter the shape of the theoretical curve, although it would of course affect its absolute values.

SUMMARY

1. A method for detecting non-lethal losses of X- and Y-chromosomes produced by irradiation of mature sperm of *Drosophila* is described in detail.

2. Losses of X and Y are found to be produced by X-raying mature sperm, and those found by the method used are non-lethal to the zygote.

3. Between 0 and 4000 r. the frequency of the above losses is, in first approximation, a linear function of the dosage, and it certainly varies less rapidly than the 1·5 power of the latter—the power found, for these dosages, in the case of gross rearrangements.

4. The mechanism of origination of these losses is interpreted as being through the occurrence of single breaks followed by lateral fusion of sister chromatid fragments, leading to formation of a dicentric chromosome which is either lost in the first change or becomes subjected to repeated breakage at successive anaphases and is ultimately lost.

5. It is suggested, as a result of comparisons of the frequency of the losses found with the amount of change of the sex ratio caused by the same dosage, that a major part of the losses are lethal to the zygote.

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INTERGENERIC HYBRIDS OF *SACCHARUM*

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(With Twenty-two Text-figures)

CONTENTS

PART I. *SACCHARUM-ERIANTHUS*

1. INTRODUCTION

In the present series of papers I propose to deal with intergeneric hybrids between *Saccharum* and other grasses.

The genus *Saccharum* consists of some ten species (Bews, 1929) distributed throughout the warmer parts of the world. The species used in the present experiments are *S. spontaneum* and *S. officinarum*, both belonging to the section *Eu-Saccharum*, and hybrids between them.

S. spontaneum is a polymorphic species. I have collected clones in India with 48, 56, 64, 72 and 80 chromosomes (Janaki-Ammal, 1936, 1939). Others from Assam and Burma had 96 and from the East Indies 112, while Bremer (1929) found forms with 80 in Celebes and the Philippines. *S. officinarum*, the cultivated sugar cane in its common forms, is octoploid, $2n = 80$. Like many important cultivated plants it is not known in the wild; its nearest wild relative is *S. robustum* $(2n = 80)$,[1] discovered by Brandes in New Guinea (1928).

The first successful cross between *S. officinarum* and *S. spontaneum* was made by Barber in 1914. Since that date a large number of hybrids and hybrid derivatives have been evolved both in India and Java (the so-called "nobilized" varieties). The first intergeneric hybrid of *Saccharum* was also made by Barber, in 1913 (Barber, 1916), when he crossed the clone "Vellai" of *S. officinarum* with the grass *Narenga narenga*. In 1927 Rumke (1934) crossed another clone "EK 28" with *Erianthus sara*. Since then a number of intergeneric hybrids of *S. officinarum* have been made (Venkatramam, 1938; Janaki-Ammal, 1938).

In 1934 I attempted a series of crosses between *S. spontaneum* and related grasses. Amongst the successful hybrids I described were those between two types of this species with 56 and 112 chromosomes and *Erianthus ravennae*, $2n = 20$ (Janaki-Ammal, Report, 1936). The first of these did not flower; the present investigation is on the second, in which the Java clone of *S. spontaneum*, "Glagah", was used as the female parent. This clone was obtained from the Pasoeroean Experimental Station and has been propagated vegetatively at the Imperial Sugar Cane Station, Coimbatore, since its introduction in 1919.

The variety "purpurascens" of *Erianthus ravennae* was used as the pollen parent. It was collected from the Punjab and was designated "*Saccharum munja*, spiny" at Coimbatore until correctly identified by Mr Hubbard at Kew in 1935.

2. METHODS

Spikelets of *S. spontaneum* selected for crossing were emasculated a day before their opening, and the rest of the spikelets removed from the "arrows" which were bagged both before and after pollination—a process which is usually thought detrimental to seed-setting in *Saccharum*. Five seedlings were obtained from this cross. The reciprocal cross set no seeds. All the F_1's were alike. My observations were made on one of the five F_1 plants—"SG 48·1" and its selfed seedlings.

[1] Brandes says $2n = 84$; material examined by me had $2n = 80$.

Root tips for chromosome counts were fixed in Allen's Bouin to contract the chromosomes and thus facilitate their counting. Root tips were immersed in crushed ice for several minutes before fixation, as this was found to give metaphase plates with the chromosomes well spaced. Pollen mother cells were fixed in 1 : 3 acetic alcohol. Acetocarmine smears were made both from fresh material and from pollen mother cells fixed in acetic alcohol and preserved in 70 % alcohol. Material thus preserved was rendered more suitable for staining in acetocarmine by immersion for a few minutes in acetic alcohol or Carnoy's fixative. Smears were made permanent by the method of McClintock (1929). Sections of root tips were cut at $10–12\mu$ and of pollen mother cells at 16μ; all sections were stained in Heidenhain's iron-alum-haematoxylin.

3. General characters of parents and offspring

Hooker in his *Flora of British India* says of *Erianthus*: "Habit and character of *Saccharum* but glume 4-awned, rarely awnless." *Erianthus ravennae*, however, differs from *S. spontaneum* in a number of characters, of which the clearest is the absence of regular internodes.

Table 1 summarizes the general characters of taxonomic value noted in the two parents and the F_1. The F_1's resembled *S. spontaneum* more

Table 1. *Comparison of characters of* Saccharum spontaneum, Erianthus ravennae *and their* F_1

| Characters	*S. spontaneum*	F_1 seedlings	*E. ravennae*
1. Stem: anatomy	Nodes and inter-nodes present	$S*$	Short rhizomes. Aerial stem developed during flowering only
2. Stem: average thickness	0·93 cm.	1·3 cm.	1·1 cm.
3. Ligule	Ovate; zone of articulation present	S	Ciliate; zone of articulation absent
4. Leaf sheath	Hairy on side of ligule	S	Bearded at insertion of leaf
5. Leaf length	40 cm.	51 cm.	44 cm.
6. Leaf width	1·9 cm.	1·8 cm.	1·3 cm.
7. Inflorescence	Subsidiary branches simple	S	Subsidiary branches compound
8. Primary rach	Hairy	S	Glabrous
9. Callus hairs	4–5 times longer than glume	3·5–4 times longer	Equal or sub-equal
10. Glume I	Membranous with coriaceous base	S	Villous dorsally
11. Glume IV	(*a*) Minute	Long	Long
	(*b*) Linear	S	Ovate
	(*c*) Ciliate	S	Non-ciliate
	(*d*) Awnless	S	Awned
12. Lodicules	Ciliate	S	Glabrous

* S = character as in *Saccharum* parent.
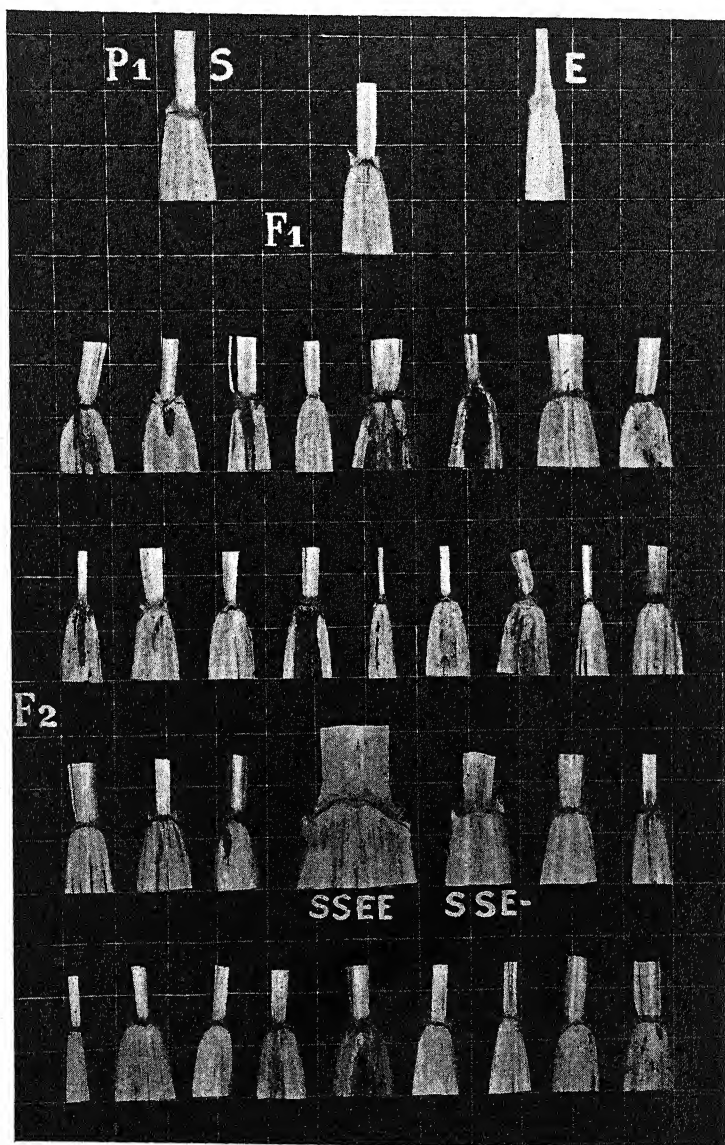
Fig. 1. Types of ligule in *S. spontaneum* and *Erianthus* P_1 and their F_1 and F_2 hybrids.

closely than *Erianthus*, but they had slightly thicker stems and longer leaves than the *Saccharum* parent and the inflorescence was on the average longer and denser. The hybrids produced abundant pollen. In the plant SG 48·1 selected for study the percentage of viable pollen was about 82 % as against 94 and 93 % noted in the *Saccharum* and *Erianthus* parents respectively. The plant set abundant seeds, even under bags.

4. Morphology of F_2 seedlings

Several hundred selfed seedlings were raised from bagged inflorescences of the *S. spontaneum* × *Erianthus ravennae* hybrid SG 48·1 in 1937; of these only fifty F_2 plants were grown for study. Owing to the drought conditions at Coimbatore in 1937 and 1938, and the salinity of the soil in which they were grown, several of the seedlings died. The remainder showed great variation in height and thickness of stem and width of leaves, some of them being much thicker and taller than any variety of *S. spontaneum*. The average width and length of the leaves was measured in the thirty-nine plants that survived. Fig. 1 shows the type of ligule in these. The frequency distribution of the seedlings in six class groups according to leaf width is recorded in Table 2. The modal class of pro-

Table 2. *Leaf width in parents and crosses*

	Leaf width in centimetres					
	1	1·5	2	2·5	3	3·5
Class of parents		*Erianthus*	*Saccharum*			
Class of F_1			F_1			
Frequency of F_2	11	19	3	2	3	1
Constitution of F_2 plants examined			$2x, SE +$		$3x, SSE -$	$4x, SSEE$

genies occurred in the 1·5 cm. group into which *Erianthus ravennae* also falls. Four plants stood out from the others by their great height. Their leaves, which exceeded 3 cm. in width, resembled those of sugar canes more than they did those of either parent or of the F_1. Fig. 2 illustrates the difference in the thickness of the stem, Fig. 3 the size of the inflorescence and spikelets in the parents F_1 and some of the F_2 seedlings. Class groups of stem diameter of forty seedlings are shown in Table 3. Plants with thicker stems had also larger inflorescences. In several individuals the subsidiary branches of the inflorescence were seen to be compound as in *Erianthus*. An awned glume was found to have segregated in the F_2. Where the awn was absent the fourth glume was generally longer than in *S. spontaneum*, as shown in Fig. 4. An important value in

comparing these hybrids is the length proportion of callus hairs to glumes. The range of this value H/G is shown in Table 4. The F_2 distribution is unimodal and covers most of the range between the parental species.
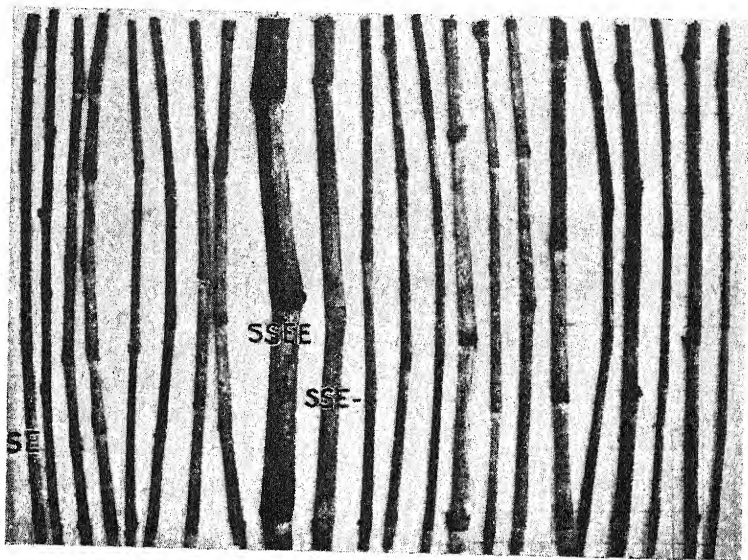

Fig. 2. Variation in stem thickness in the F_2 generation of *Saccharum-Erianthus* hybrids

Table 3. *Stem diameter in parents and crosses*

	Stem diameter in centimetres				
	0·5	1	1·5	2	2·5
Class of parents Class of F_1		*Erianthus*	*Saccharum* F_1		
Frequency of F_2	2	31	3	3	1
Constitution of F_2 plants examined		2x, SE +		3x, SSE –	4x, SSEE

Table 4. *Distribution of* H/G *ratio in* S. spontaneum *and* E. ravennae *parents and hybrids*

Class	Class value	Class : parents and F_1	Frequency F_2
0·8–1·2	1	*E ravennae*	—
1·3–1·7	1·5	—	1
1·8–2·2	2	—	1
2·3–2·7	2·5	—	6
2·8–3·2	3	—	4
3·3–3·7	3·5	F_1	15
3·8–4·2	4		2
4·3–4·7	4·5	*S. spontaneum* "Glagah"	—
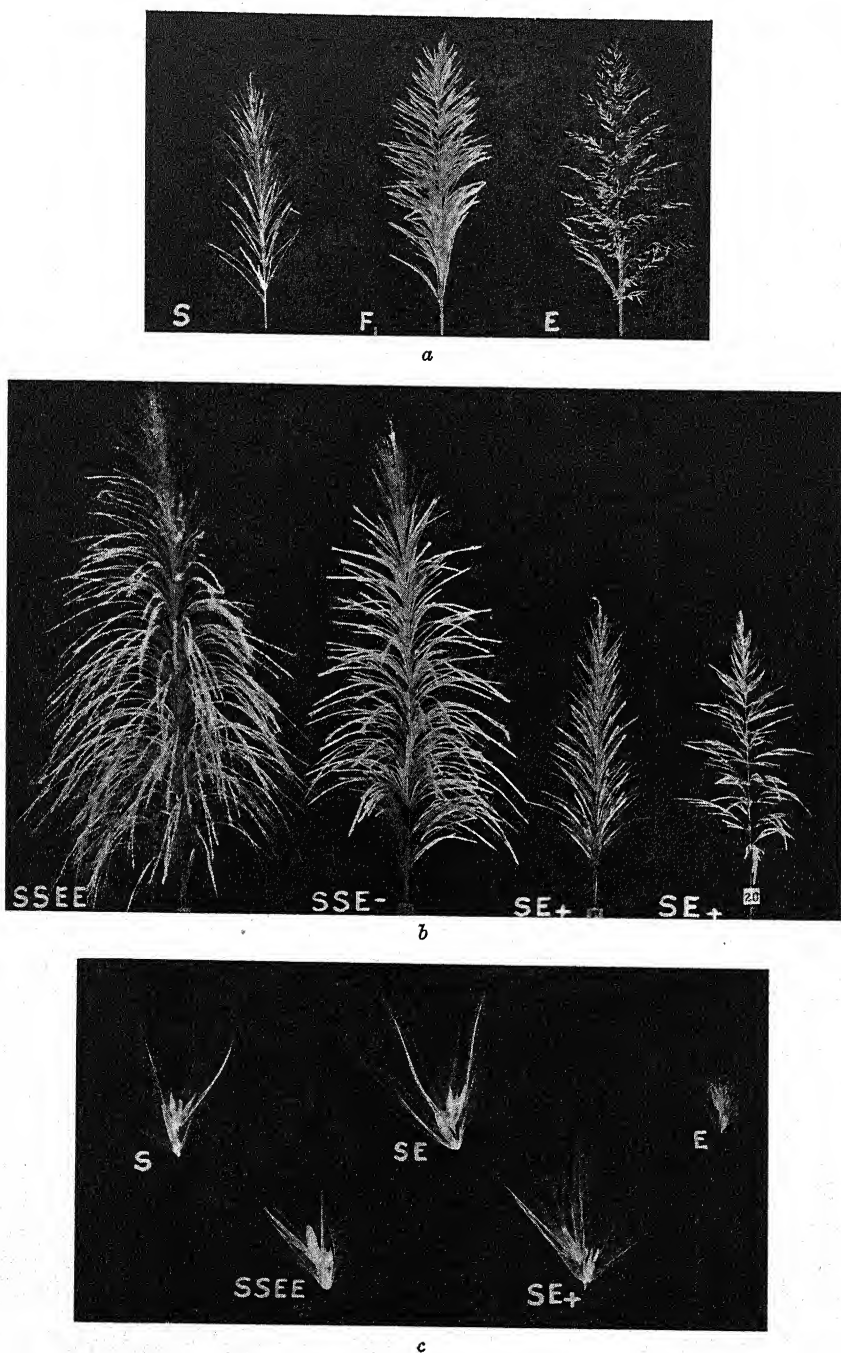
Fig. 3. *a*, the inflorescence of *S. spontaneum* (*S*), *Erianthus* (*E*) and their *F*₁ hybrid.
b, relative size of arrows in *F*₂ of tetraploid (*SSEE*), triploid (*SSE*) and diploid (*SE* +).
c, spikelets of *Saccharum* (*S*), *Erianthus* (*E*), their *F*₁ hybrid (*SE*) and two *F*₂ plants.

The F_2 seedlings varied a great deal in the degree of anthesis. In at least four of the seedlings that flowered there was total absence of anthesis. This was generally associated with low percentage of viable pollen.
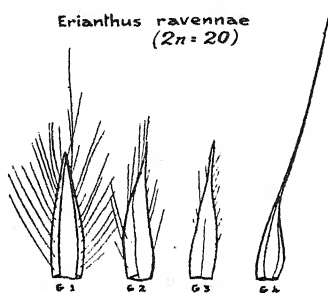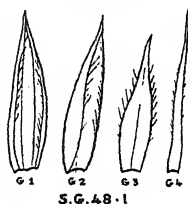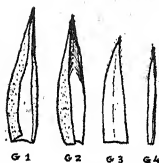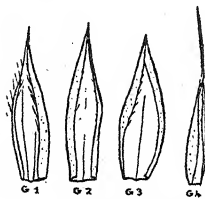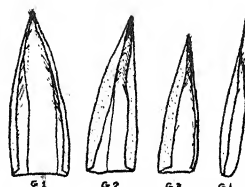
Fig. 4. Types of glume in spikelets of *Saccharum, Erianthus* and their F_1 and F_2 hybrids. The awned fourth glume, though not present in the F_1, appears in some of the F_2 seedlings.

5. CHROMOSOME NUMBERS IN PARENTS, F_1 AND F_2

Root tips of *S. spontaneum* "Glagah" showed 112 chromosomes, as found by Bremer (1923). The somatic number of *Erianthus ravennae* is 20. In the variety "spiny" used in the present cross there was a small extra fragment. Four selfed seedlings of this plant showed the 20 chromosomes only. The basic number in the genus *Erianthus* is 10, and *E. ravennae* is therefore a diploid species.

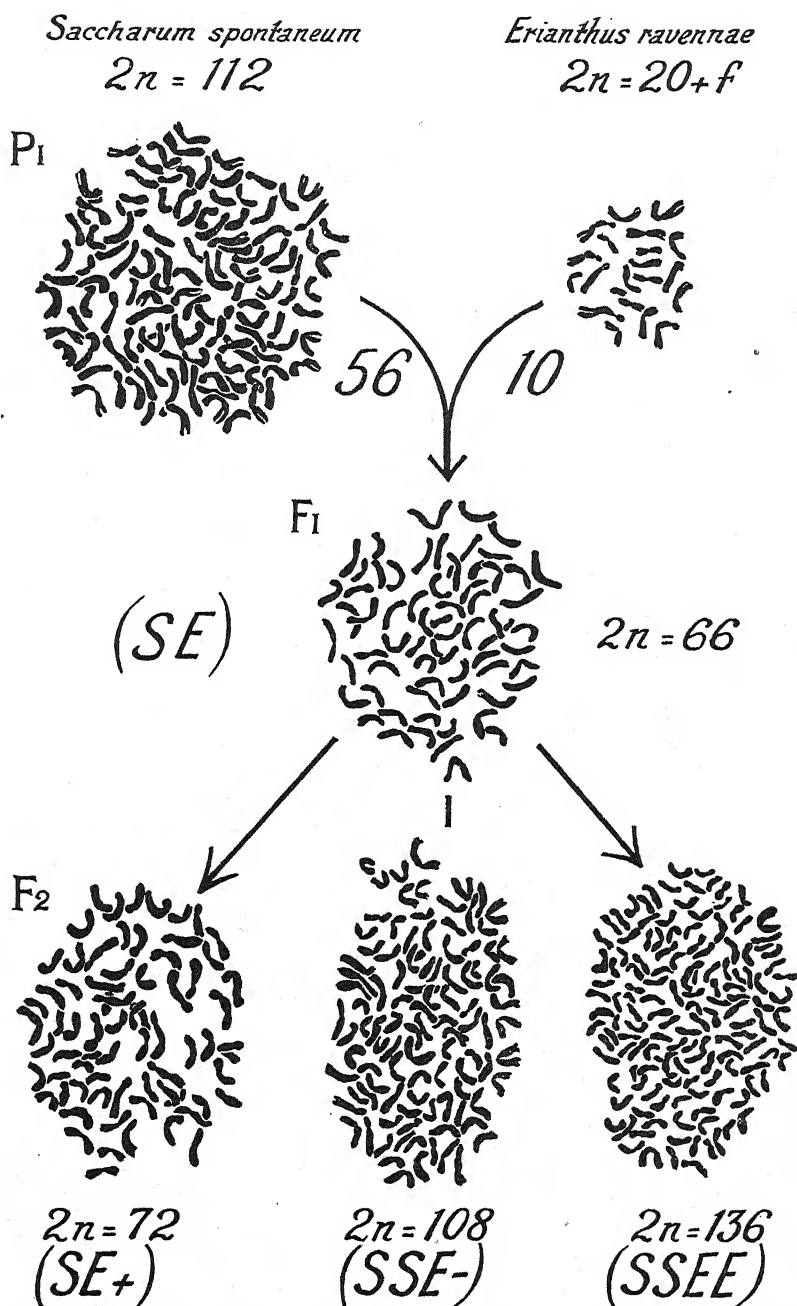All the five F_1 hybrids examined had $2n = 66$, the sum of the haploid numbers of the parents.

Fig. 5. Somatic metaphase in root tips of *S. spontaneum*, *Erianthus ravennae* and their *F*₁ and *F*₂ hybrids. ×2000.

The following are the chromosome numbers of the sixteen F_2 seedlings examined:

Chromosome no....	67	68	69	70	71	72	73	74	75	76	104	106	108	136
No. of plants	1	1	—	1	1	2	3	1	—	2	1	1	1	1
Presumed constitution					$SE+$						$SSE-$		$SSEE+$	

Twelve of the sixteen seedlings examined had a chromosome number between 67 and 76, that is, close to that of the F_1 hybrids. Three plants had 104–108 chromosomes and are therefore "triploids", SSE, in relation to those in which the chromosome number ranged from 67 to 76. A single plant had $2n = 136$ (Fig. 6) and would on the same evidence be considered a "tetraploid", $SSEE$. The higher chromosome numbers go with the larger size of stem, leaves and inflorescence.

The chromosomes of the parents, F_1 and three types of F_2 are shown in Fig. 5.

6. Meiosis in the parent species

The 112 chromosomes of *S. spontaneum* "Glagah" associate as 56 bivalents at diplotene (Fig. 6a). The number of chiasmata at this stage varies from one to two per bivalent. Fig. 6b represents the 56 bivalents
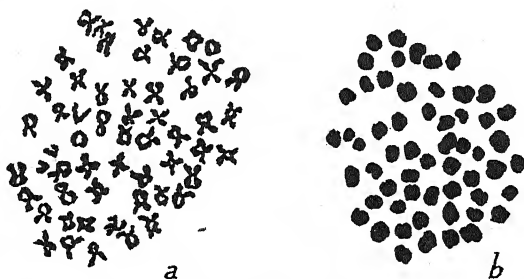

Fig. 6. Chromosome association in *S. spontaneum* "Glagah". *a*, diplotene; *b*, metaphase.
× 1800.

at metaphase. Reduction division in this plant, which has also been dealt with by Bremer, shows regular distribution of the 56 bivalents during anaphase.

Fig. 7 *a–c* represents the stages of meiotic division in the male parent, *Erianthus ravennae*. The chromosomes associate as 10 bivalents, the number of chiasmata varying from two in the short chromosomes to three in the longer ones. The single centric fragment is not included in the metaphase plate and is lost in the cytoplasm. It is probably eliminated in gamete formation.
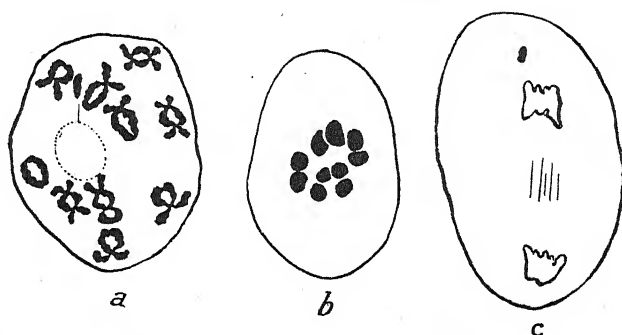
Fig. 7. Meiosis in *Erianthus ravennae*. *a*, diplotene; *b*, metaphase; *c*, telophase, with the fragment excluded. ×2000.

7. MEIOSIS IN THE F_1 HYBRID

The F_1 hybrid flowered abundantly. Pollen mother cells at diakinesis showed that the 66 chromosomes associate into bivalents, trivalents and quadrivalents (Fig. 8*a*). Table 5 gives the configurations noted in ten

Table 5. *Degree of association of chromosomes in* F_1

Configurations				
IV	III	II	I	Cells
1	1	26	7	5
1	2	24	8	1
1	2	25	6	1
2	2	23	6	1
2	2	22	8	1
2	1	24	7	1

cells in which all the chromosomes were present. The large number of bivalents (22 to 26) present in the hybrid shows that the chromosomes derived from the haploid complement of the *S. spontaneum* are capable of pairing amongst themselves (autosyndesis) like the *Tripsacum* chromosomes in the cross between *Zea* and the tetraploid form of *Tripsacum dactyloides* (Mangelsdorf & Reeves, 1932).

The number of univalents varied from six to eight. These univalents probably represent the unpaired *Erianthus* chromosomes. The frequent presence of seven univalents associated with a single quadrivalent and a trivalent indicates that at least three of the chromosomes of *Erianthus* pair with those of *Saccharum*, forming the multiple associations noted. Not infrequently the number of these multiple associations is greater than one. An increase in their number is associated with a decrease in the number of bivalents rather than with any appreciable change in the

number of univalents. We might infer from this that the gametic comple-
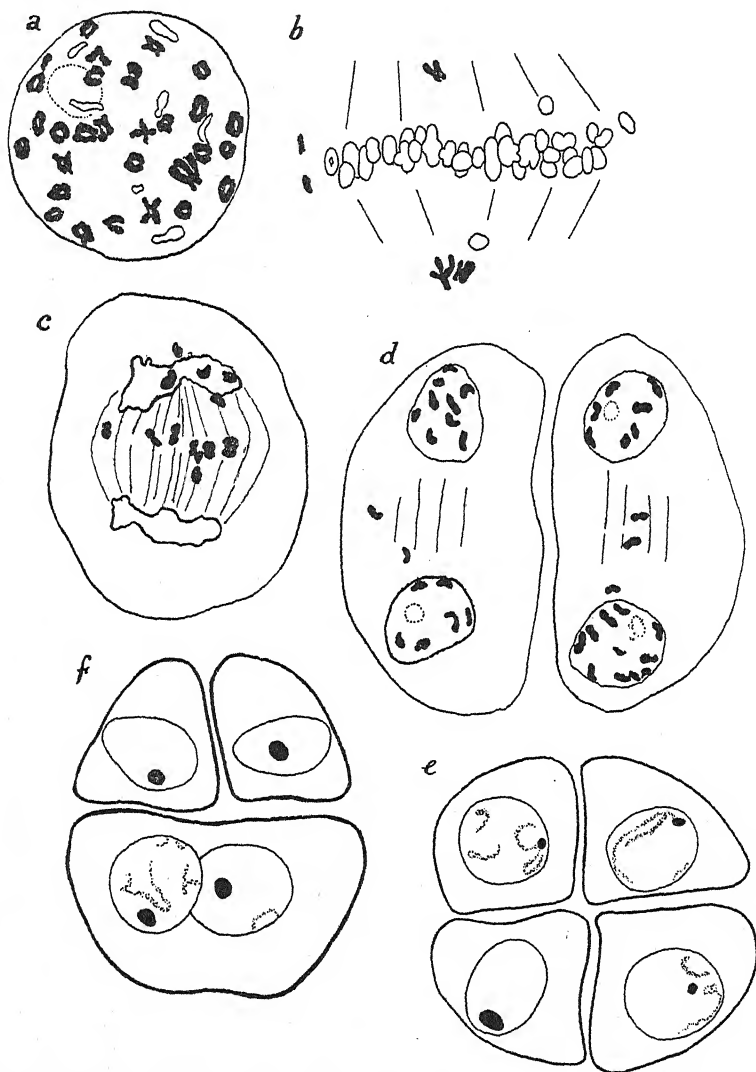ment of *S. spontaneum* present in the hybrid is capable of forming higher

Fig. 8. Meiosis in *Saccharum-Erianthus* hybrid. *a*, diakinesis. *b*, first metaphase. *c*, telo-
phase of first division, showing univalents dividing at the equator. *d*, telophase of
second division, showing lagging chromosomes. *e*, normal tetrad. *f*, abnormal tetrad
with binucleate dyad. (*a*, *b*, *c* and *d*, ×1800; *e* and *f*, ×1080.)

configurations by autosyndesis than it does in the polyploid parent. In
this respect the *S. spontaneum* × *Erianthus* hybrid is similar to the

diploid-hexaploid hybrid *Lolium perenne × Festuca arundinacea* in which Peto (1934) found trivalents, quadrivalents and quinquevalents.

Differential condensation of chromosomes was noticed in some of the cells. Fig. 8*b* shows a metaphase plate in which two bivalents seem to be at an earlier stage than the rest of the chromosomes. A variable number of univalents are seen to divide on the spindle, after the bivalents separate to opposite poles (Fig. 8*c*). At the second metaphase a number of daughter univalents are seen to lag and segregate at random without splitting. Some of these do not reach the poles before the nucleus is reconstructed (Fig. 8*d*). This variable segregation is responsible for the occurrence of F_2 seedlings with numbers ranging from 67 to 76. The development of the pollen grain was normal in a large percentage of the cells examined, but occasionally tetrads with two-nucleate cells and dyads were found (Fig. 8*f*). These would give rise to unreduced pollen grains. The occurrence of unreduced mother cells giving rise to unreduced embryo sacs is a common feature in *Saccharum* (Janaki-Ammal, 1939; S. Narayanaswamy, 1940). Fertilization of these diploid eggs by haploid and diploid pollen grains accounts for the occasional "triploids" and "tetraploids" found amongst the F_2 seedlings.

8. MEIOSIS IN F_2 SEEDLINGS

Fig. 9 *a, b, c*, represents the association of chromosomes in three of the "diploid" seedlings. The number of univalents was variable in all the plants studied. Chromosome association was chiefly in the form of bivalents with an occasional quadrivalent. In the "triploid" with 108 chromosomes I found occasional sexivalents besides bivalents (Fig. 9*d*).

Fig. 9 *e* and *f* illustrates the chromosome configuration in pollen mother cells of the single "tetraploid" plant SG 100·33 during diakinesis and metaphase. The chromosomes associate as bivalents, quadrivalents and occasionally sexivalents. The number of univalents was considerably less than in the diploid plants, and both first and second meiotic divisions were more regular than in these.

9. SUCROSE CONTENT OF HYBRIDS

Crossing *S. officinarum* with *Erianthus* results in hybrids with a reduced sugar content (Rumke, 1934). The F_1 hybrid between *S. spontaneum* "Glagah" and *Erianthus* also showed considerable reduction; the purity of the juice was also lowered. Table 6 gives the analysis of sugar in the cross as well as in five of the F_2 hybrids, two diploid, two triploid, and one tetraploid. It will be seen that, whereas the diploid hybrids were

approximately as sweet as the F_1, the triploid and tetraploid plants showed a considerable increase in the percentage of sugar present. This is analogous to the findings in an autotriploid of *S. spontaneum* examined by the writer (Janaki-Ammal, 1939). It can be inferred from these
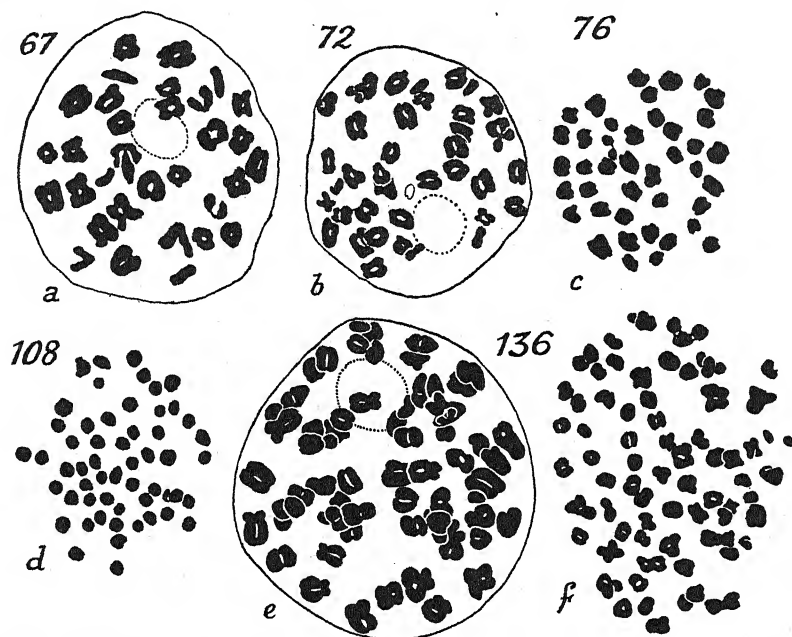

Fig. 9. Association of chromosomes in F_2 seedling. *a, b, c,* "diploids" with 67, 72 and 76 chromosomes. *d,* metaphase in triploid with 108 chromosomes. *e, f,* diakinesis and metaphase in tetraploid, $2n = 136$, showing quadrivalents and sexivalents. $\times 1800$.

Table 6. *Sugar analysis of* Saccharum × Erianthus *hybrids and* Saccharum *parent*

	Sucrose %	Purity %
S. spontaneum "Glagah"	7·93	60·5
S. spontaneum × *Erianthus* F_1	3·64	36·5
F_2's, diploid: S.G. 100·3	3·51	34·5
S.G. 100·16	2·33	27
F_2's, triploid: S.G. 100·5	5·45	42·4
S.G. 100·35	6·30	55·7
F_2, tetraploid: S.G. 100·33	6·51	58·3

observations that polyploidy results in an increase in sugar production in both *S. spontaneum* and its hybrids. The tetraploid F_2's, however, still lack the content of the *Saccharum* parent and can be used for commercial purposes only through further crossing with the cultivated sugar cane, *S. officinarum.*

10. Summary

1. The Javanese variety "Glagah" of *Saccharum spontaneum*, $2n = 112$, when crossed with *Erianthus ravennae*, $2n = 20 + f$, gave fertile hybrids with 66 chromosomes.

2. The F_1 hybrids resembled the two parents in proportion to their chromosome contributions (56 and 10).

3. The F_2 seedlings fell into three groups in regard to their chromosome numbers:

Diploids, $SE+$	68–76
Triploids, $SSE-$	104–108
Tetraploid, $SSEE+$	136

4. The diploid seedlings were the great majority. They showed segregation of the *Erianthus* characters—presence of awn and compound inflorescence—and a unimodal distribution of the length proportion of callus hairs to glumes. The triploid and tetraploid seedlings had thicker stems, wider leaves and a larger inflorescence than the diploids.

5. The sugar content of the *Saccharum* parent was greatly reduced in the diploid seedlings and slightly reduced in the triploids and tetraploid.

6. In the F_1 hybrid the gametic complement of *S. spontaneum* is capable of pairing by autosyndesis and may form higher configurations than in the parent. Some of the *Erianthus* chromosomes join with those of *Saccharum* to form trivalents and quadrivalents. The others are unpaired, and are lost or distributed at random in meiosis. Binucleate tetrads and dyads are formed by suppression of one division. Chromosomes condense differentially in some of the pollen mother cells.

7. At meiosis in the diploid F_2 hybrids, quadrivalents and many univalents are present and the division is irregular. In the tetraploids, though a few quadrivalents and even sexivalents are present, there are fewer univalents and the division is more regular.

Part II. *SACCHARUM-IMPERATA*

1. Introduction

The true octoploid species *S. officinarum*, when used as the female parent in intergeneric crosses, has given economically disappointing results. However, when Thomas and Venkatraman (1930) and Bourne (1935) crossed the hybrid cane of Java, "POJ 2725", $2n = 106$, with *Sorghum* they obtained some seedlings of value, together with large numbers considered useless to the sugar cane breeder. Since then, "POJ 2725" and another Java cane, "POJ 213", $2n = 124$, have been extensively used in

breeding and spectacular results have been obtained with widely differing genera of grasses, including the bamboo (Venkatraman, 1937). In the present experiment I have used the cane "POJ 2725" in crosses with *Imperata*.

The grass *I. cylindrica* has a wide distribution in both the Old and the New World. It is a troublesome weed of cultivated land. There are several ecotypes, ranging from dwarfs of a few inches in height to swamp forms over 9 ft. tall with inflorescences up to 20 in. in length. The form I used for hybridization with the sugar cane was that known in Malay as "alang-alang", with a chromosome number of 20. It had a small inflorescence (Fig. 10). Bremer, who examined pollen mother cells (1925), found regular pairing and meiosis in the plant. Its gametic complement is therefore 10. The seed was supplied by the Department of Agriculture, Kuala Lumpur, Malaya.

In 1935 I pollinated an arrow of POJ 2725 with pollen of *I. cylindrica*, under a bag, and obtained thirty-five seedlings. All of these looked very much like sugar canes, though a few were inclined to dwarfness. From an unpollinated control arrow under a bag I obtained a single seedling in 1935 and four seedlings in 1936. The present investigation concerns some of the surviving seedlings. Only fourteen were examined cytologically.

2. Methods

Canes of POJ 2725 were transported from the field to a pollinating shed when about to arrow. The arrows were bagged both before and after pollination. Root tips were fixed in Bouin's fixative and La Cour's 2BD after pre-treatment with ice. Pollen mother cells were fixed in acetic alcohol 1 : 3.

3. Cytology of the *Saccharum* parent; POJ 2725

The parentage of POJ 2725 is given in Table 7. It was produced at the Pasoeroean Station in Java by Jeswiet and has been described as "the product of the third nobilization of *S. spontaneum* Glagah".

When *S. officinarum* ♀ is crossed with *S. spontaneum* ♂, the F_1 in all cases examined is the result of the fertilization of an unreduced egg of the first species by a reduced pollen grain of the second (Bremer, 1929; Dutt & Subba Rao, 1933). It has the composition *OOS* where *O* and *S* stand for the complements of the two species. In back-crossing the hybrid "Kassoer", of this constitution, as male to *S. officinarum* ♀, fertilization was again confined to 2n eggs, so that POJ 2364, the female parent of the

Imperata cylindrica (⅔ nat. size.)

F_1
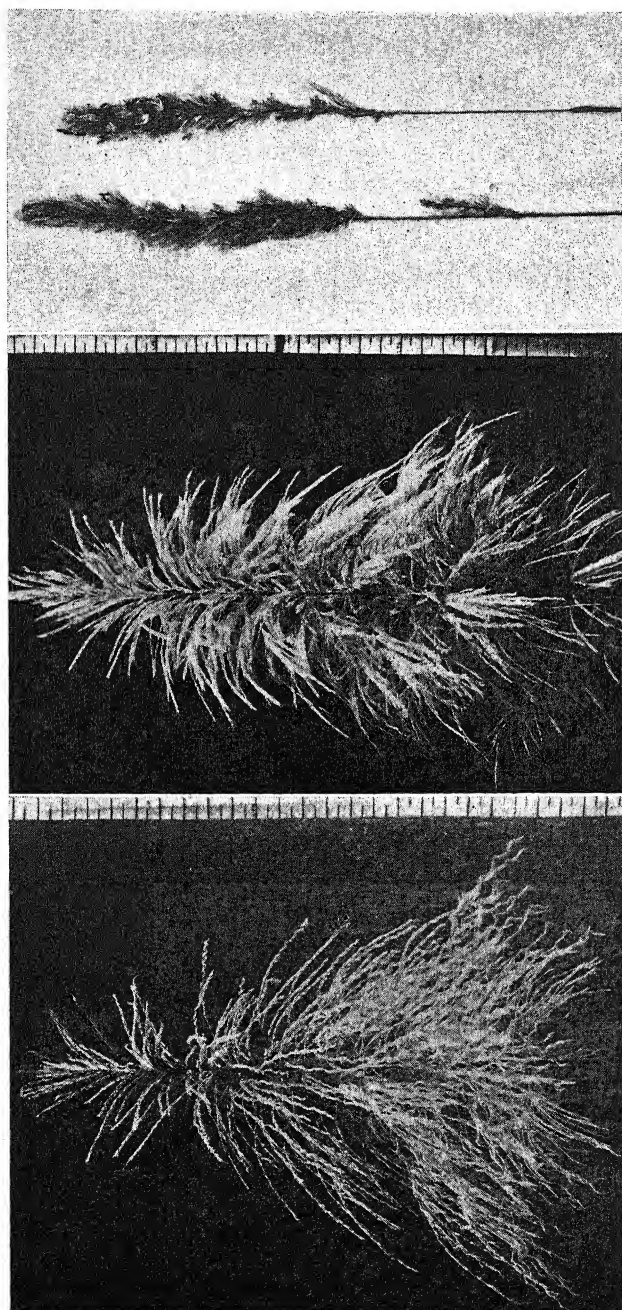
POJ 2725

Fig. 10. Inflorescence of POJ 2725, *Imperata cylindrica* and F_1.

Table 7. *Origin of the clone* POJ 2725

S. officinarum ♀   ×   *S. spontaneum* ♂
"Black Cheribon", 2n = 80   "Glagah", 2n = 112
(OO)   (SS)

S. officinarum ♀   ×   "Kassoer" ♂
POJ 100, 2n = 80   2n = 136
(OO)   (OOS)

POJ 2364 ♀   ×   *S. officinarum* ♂
2n = 148   EK 28, 2n = 80
(OOO + S/2)   (OO)

POJ 2725
2n = 106
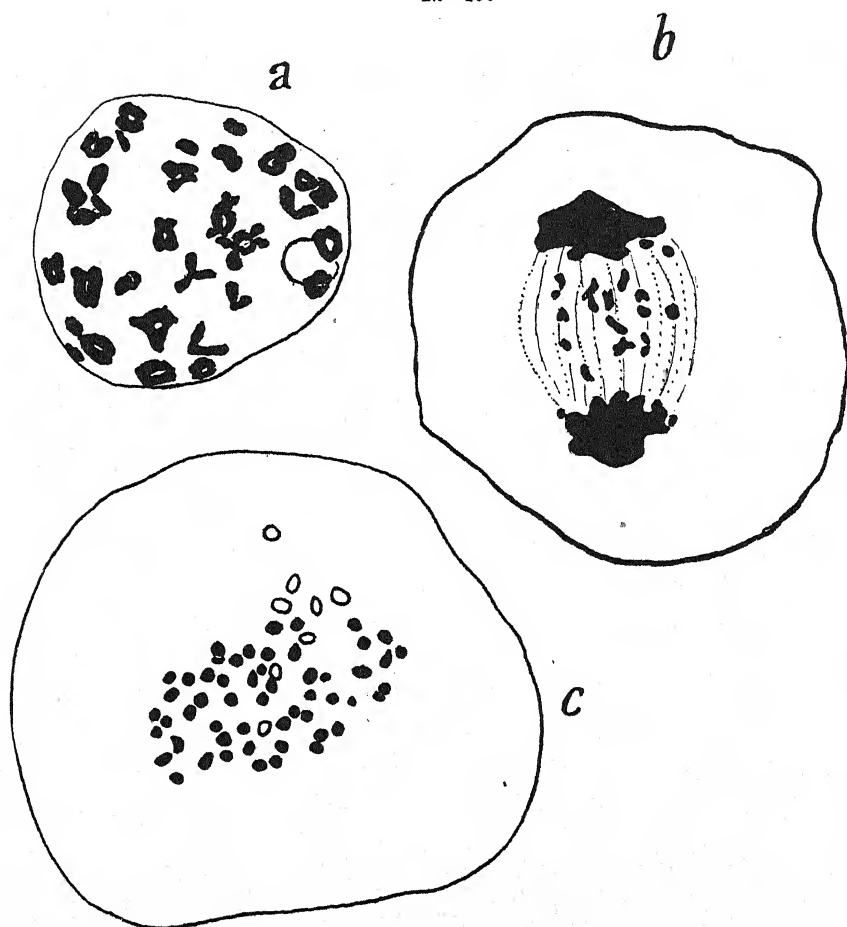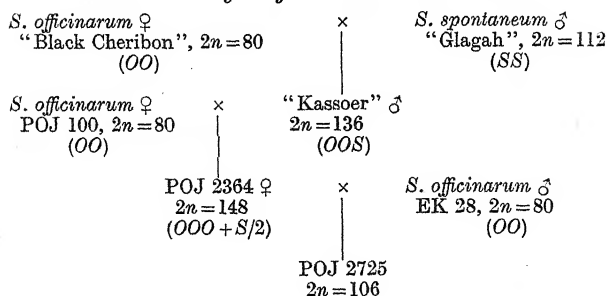
Fig. 11. Meiosis in POJ 2725. *a*, pollen mother cell at diakinesis showing multivalent and univalent association of chromosomes × 2400. *b*, anaphase of first division. *c*, metaphase in pollen grain with 62 chromosomes × 1800.

cane POJ 2725, may be considered as a triploid *S. officinarum* plus about half the gametic complement of *S. spontaneum*.

As would be expected, meiosis in POJ 2364 is very irregular. When backcrossed with *S. officinarum* it produced seedlings having a variable number of chromosomes, 106–120. According to Bremer (1928) POJ 2725 has 106–7 chromosomes; I was able to count 106 only. The chromosomes in pollen mother cells of POJ 2725 at diakinesis associate as bivalents, trivalents and quadrivalents (Fig. 11*a*). A number of univalents were also regularly observed, and many divided at first anaphase (Fig. 11*b*). Meiosis is consequently irregular. The irregular distribution of chromosomes gives gametes with variable numbers; 62 were found at metaphase in a pollen grain (Fig. 11*c*). Hence it seems unlikely that POJ 2725 will contribute its exact haploid number, 53, to any of its hybrids.

The percentage of viable pollen was about 21 %. Anthesis was poor and variable. In a sample from one arrow 2 % of the anthers had dehiscence pores. Selfing or parthenogenesis is therefore responsible for the few seedlings obtained under bag in 1936–8.

4. TRUE AND FALSE HYBRIDS

The following are the chromosome numbers of the fourteen seedlings:

	Chromosome no.	No. of plants	Origin	Presumed constitution	Pollen fertility
I	106	2	Vegetative embryo	SS	23–35 %
II	108 110 112	3 2 1	Sexual or diploid parthenogenesis	$SS+$	0·0–7 %
III	120 130 132 134	1 1 1 2	True F_1	SSI	50–80 %
IV	156	1	Triploid self	SSS	Non-flowering

It will be seen that they fall into four groups, of which representative types are shown in Fig. 12. First, there are those that have the same number as POJ 2725. These also resemble it closely in vegetative characters and have the same pollen fertility, 23–35 %. They are in all probability vegetatively apomictic plants and could be considered as clones of the mother plant (Fig. 13, type A).

To the second group belong plants in which the chromosome number is slightly greater than in POJ 2725. They show segregation of characters and are very highly sterile, the pollen fertility being 0–7 %. From the evidence of their chromosome number and morphology, they would appear to be either true selfs or parthenogenetic plants developed from unre-
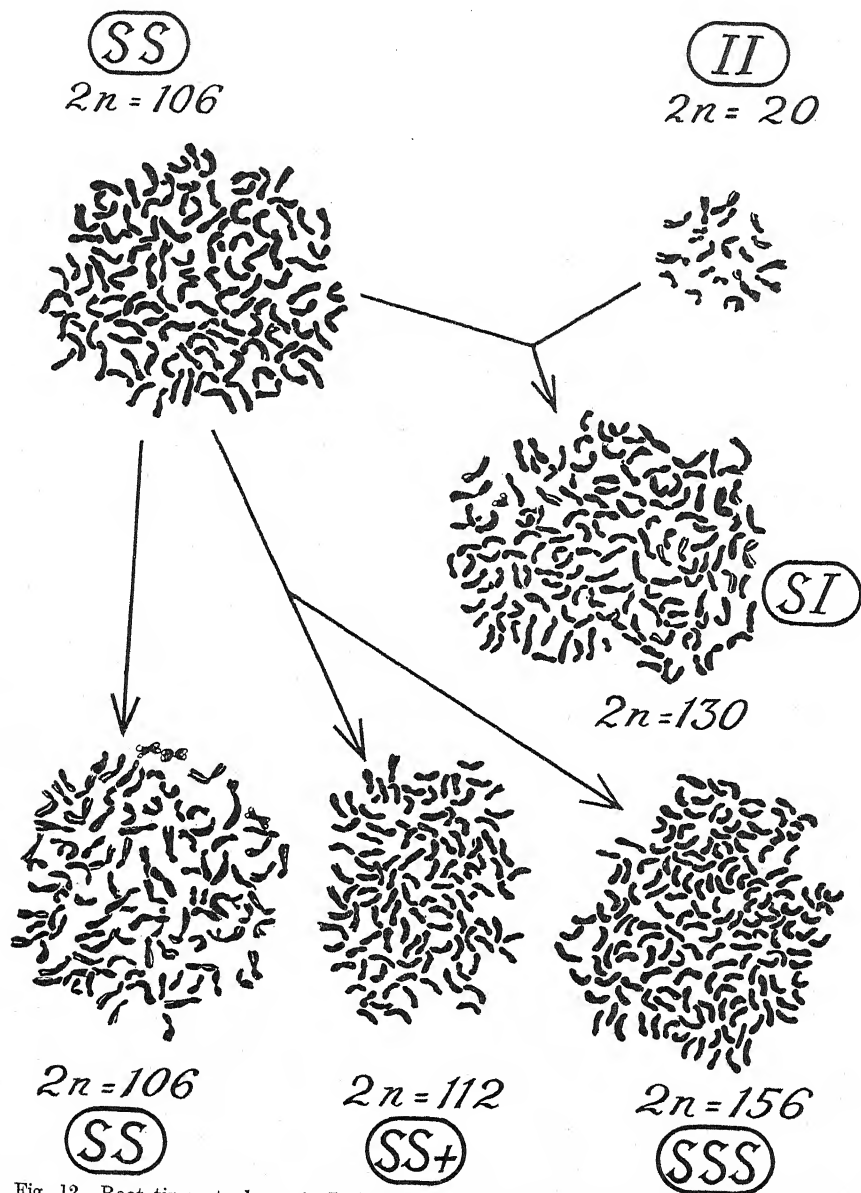
Fig. 12. Root tip metaphases in POJ 2725. (*SS*) *Imperata cylindrica* (II) and types of seedlings obtained in the cross. ×2000.

duced gametes (types B and D in Fig. 13). Bourne (1935) found that about 1 % of the seedlings produced by pollinating this cane with *Sorghum* are of a maternal type. Venkatraman & Thomas (1930) have made no reference to the existence of these types amongst their POJ 2725 *Sorghum* hybrids.

In the third group are plants with 120–134 chromosomes (type E, Fig. 13). These numbers represent the sum of the chromosomes from unreduced gametes of POJ 2725 and from the haploid gamete of *Imperata*.
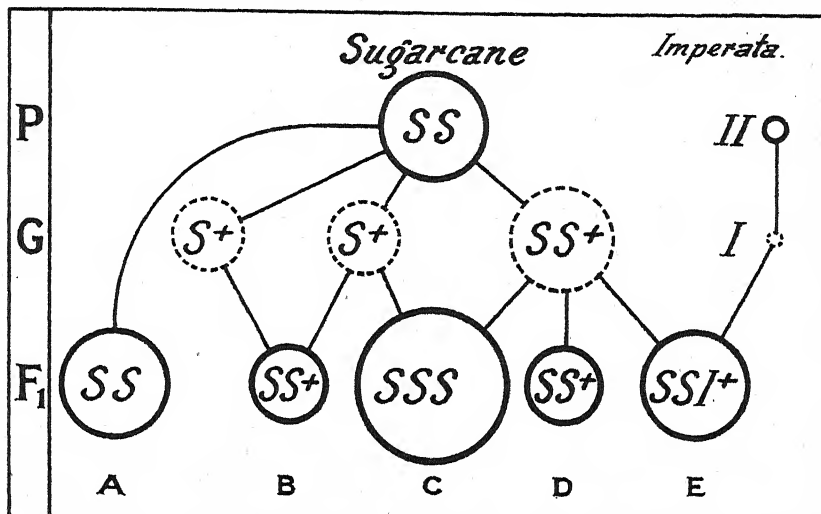

Fig. 13. Diagram of the genetic composition of seedlings from the cross POJ 2725 × *Imperata*.

They represent the only true hybrids of *Saccharum* and *Imperata*. These seedlings were highly fertile; they resembled sugar canes very closely, though they are more of the medium-cane type than the mother plant. A few showed multiple bud formation and the characteristic tillering of *Imperata*, but no importance was attached to these characters as they appear in hybrid sugar canes also.

The spikelets of *Imperata* differ from those of *Saccharum* in having fine hairs on the first and second glume. This character was found in some of the hybrids. The number of stamens also varied from three to four in odd spikelets of an inflorescence.

Meiosis in pollen mother cells of one hybrid with 120 chromosomes showed no configurations higher than bivalents (Fig. 14a). A variable number of univalents were present. These generally divided at the equator of the spindle after the bivalents had separated to the poles

(Fig. 14 *b*), and were nearly always incorporated in the daughter nuclei. It is not possible to say whether they are unpaired chromosomes of *Imperata* or of *Saccharum*. Second division was fairly regular. The
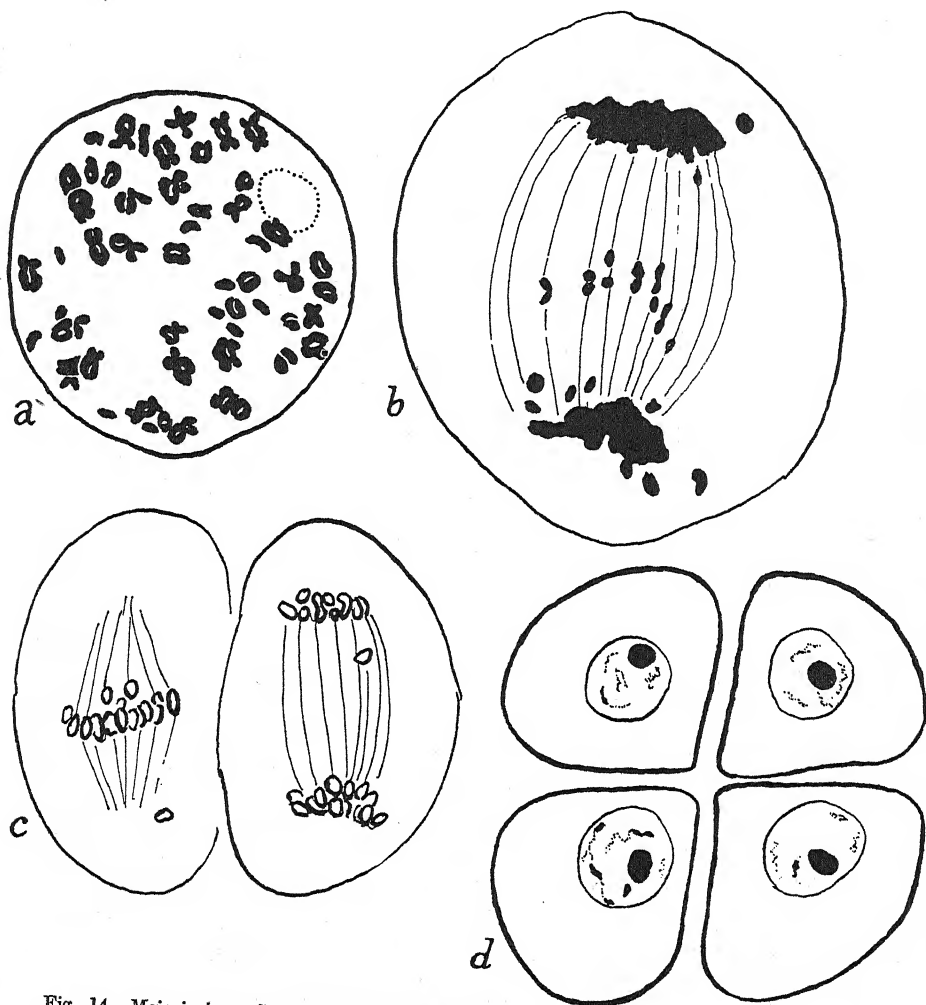

Fig. 14. Meiosis in a *Saccharum-Imperata* hybrid. *a*, diakinesis in pollen mother cell. *b*, first anaphase. *c*, second anaphase. *d*, tetrad formation. × 2000.

divided univalents were segregated at random to the poles (Fig. 14*c*), and normal tetrads formed (Fig. 14*d*).

All the hybrid F_1 plants were fertile, pollen fertility being as high as 80 % in some. An interesting series of F_2's was obtained from this plant,
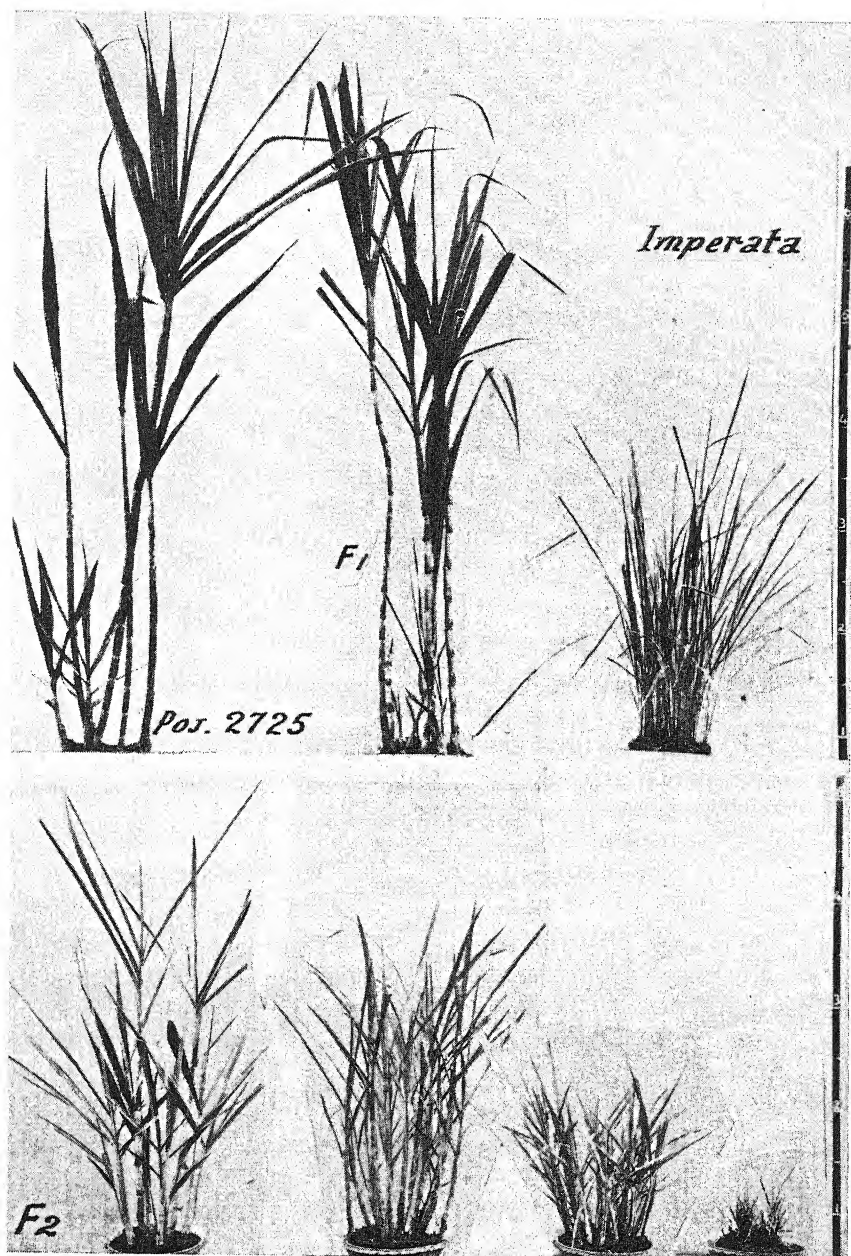
Fig. 15. General habit of POJ 2725, *Imperata cylindrica* and their F_1 and F_2 hybrids.

some of which were similar to *Imperata* in grass habit and size of leaf (Fig. 15). It will be seen then that when POJ 2725 is crossed with *Imperata*, hybrids are produced through the agency of unreduced embryo-sacs. This elimination of normally reduced eggs is responsible

(1) for the extremely few seedlings produced,
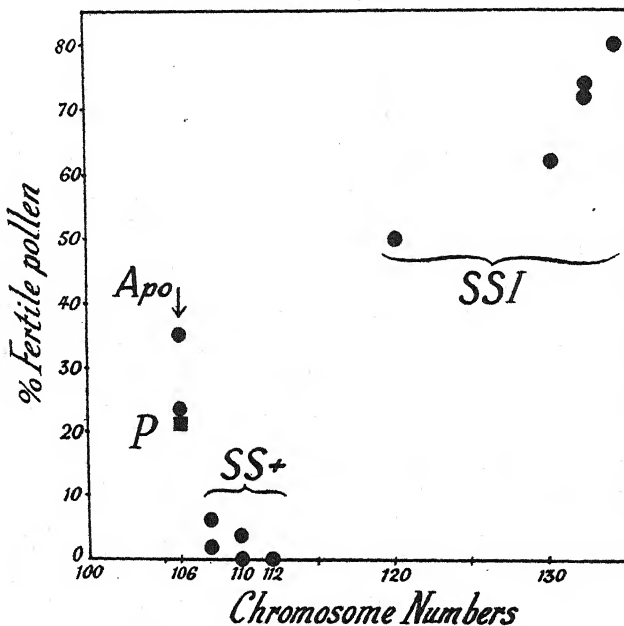(2) for the sugar-cane like characters of the hybrids.

Fig. 16. Graph showing relative pollen fertility of POJ 2725 (*P*) and its apomictic (*Apo*), parthenogenetic or selfed (*SS* +), and hybrid (*SSI*) seedling.

The proportion of chromosomes of sugar cane to *Imperata* in the seedlings is 11 : 1 in the hybrids with the lowest number, and 12·4 : 1 in that with the highest number, 134.

The single plant with 156 chromosomes in the population may be considered as a "triploid" POJ 2725 (type C in Fig. 12). Unlike other triploids it is much smaller than the parent. It probably suffers from the disadvantage of having too many chromosomes for the size of the cell. So far it has not flowered.

Fig. 16 gives the pollen fertility of the three classes of seedlings.

5. Sucrose content of the seedlings

Table 8 gives the sugar analysis of twenty-eight out of the thirty-five seedlings obtained by pollinating POJ 2725 with *Imperata*. The canes were analysed before they were fully mature, but the percentage of sugar present in the seedlings even at this stage is high enough to indicate the economic value of this cross.

Table 8. *Sugar analysis of twenty-eight seedlings produced by pollinating* POJ 2725 *with* Imperata

Seedling no.	Brix	Sucrose	Purity
63·1	15·98	13·27	83·00
63·2	19·99	17·21	86·10
63·3	16·88	13·68	81·00
63·4	17·38	13·67	78·00
63·5	19·19	16·08	83·80
63·6	18·29	14·80	80·90
63·7	21·40	16·14	75·40
63·8	19·04	15·82	83·10
63·9	20·49	17·79	87·00
63·10	15·21	11·95	78·60
63·11	16·14	13·49	83·60
63·12	15·64	12·18	79·50
63·13	15·67	12·14	77·50
63·15	18·07	15·19	84·10
63·21	17·27	14·29	82·80
63·23	19·20	17·12	89·20
63·24	17·53	15·20	86·70
63·25	21·25	18·60	89·60
63·26	19·75	17·63	89·20
63·27	18·14	15·80	87·10
63·28	17·63	14·79	83·70
63·29	19·04	17·28	90·70
63·30	17·23	14·08	81·70
63·31	15·93	12·77	80·20
63·32	19·44	16·87	86·80
63·33	17·73	15·34	86·50
63·34	19·24	17·56	91·30
63·35	20·25	18·19	89·80

Further analysis of some of the more promising seedlings showed that they were capable of still better performance. Thus the hybrid 63·32, $2n = 132$, which has an excellent erect habit and good tillering, had 19 % sugar when fully mature. The percentage was even higher in some others, but these were not so good from the agricultural point of view.

An interesting point is that the asexually produced seedlings SS with 106 chromosomes, whose genetic composition should be identical with that of POJ 2725, had a lower sucrose content than the sexually and subsexually produced SS + type—probably because they are late in maturing.

The level of performance reached by the true hybrids *SSI* is as good as that of the selfed or diploid parthenogenetic seedlings, which is certainly due to the high proportion of sugar cane to *Imperata* chromosomes in this cross (12 : 1).

In Fig. 17 I have correlated the sucrose percentage with the genetic constitution of seedlings examined cytologically.
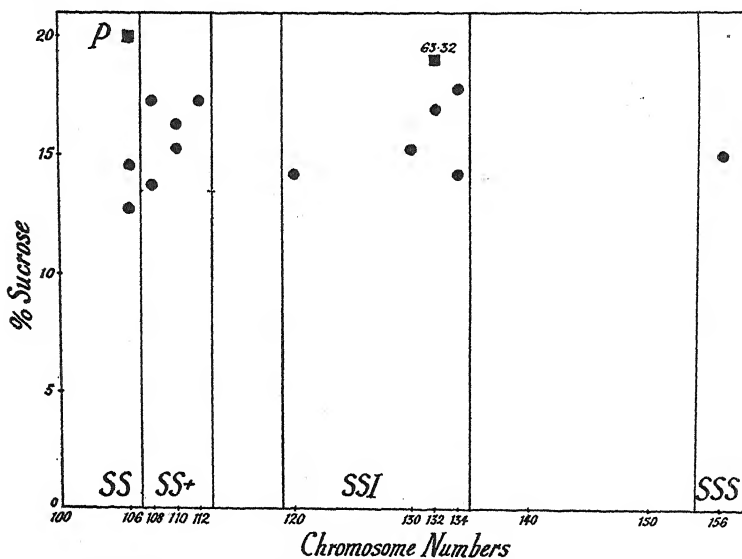

Fig. 17. Chromosome numbers and percentage sucrose in "true" and "false" hybrids of POJ 2725. The squares indicate maximum yield at full maturity, the circles yield of canes not yet fully matured.

6. SUMMARY

1. By pollinating an arrow of the hybrid cane POJ 2725 with pollen of *Imperata cylindrica*, thirty-five seedlings of fairly high sucrose content were obtained.

2. Cytological analysis of the seedlings showed them to be of four types:

(i) Vegetative seedlings, *SS*, $2n = 106$.

(ii) Selfed or diploid parthenogenetic seedlings, *SS+*, $2n = 108-112$.

(iii) Triploid self, *SSS*, $2n = 156$.

(iv) True *Saccharum-Imperata* hybrids, *SSI*, $2n = 120-134$.

3. The vegetative seedlings resembled POJ 2725 in vegetative characters and degree of pollen sterility; parthenogenetic and selfed seedlings were completely sterile and the true hybrids highly fertile.

4. Elimination of all but unreduced eggs is responsible for the small number of F_1 seedlings produced. The high proportion of *Saccharum* chromosomes accounts for the predominance of *Saccharum* characters in the hybrids.

5. The "triploid" selfed seedling is much smaller than the parent, and has not flowered.

6. F_2 seedlings of the true *Saccharum-Imperata* hybrids show segregation of *Imperata* characters.

Part III. *SACCHARUM-ZEA*

1. Introduction

Crosses described in Parts I and II of this paper involved *S. spontaneum* or its cultivated derivatives. We now turn to a cross involving *S. officinarum* proper.

As mentioned above, Barber in 1913 produced a hybrid of *S. officinarum* "Vellai" with the grass *Narenga narenga*. The clone "Vellai" is male sterile owing to the suppression of anthesis, and this sterility is a genotypic character not due to irregular meiosis. The same character is found in other clones, including the Black Cheribon of Java. "Vellai" has been crossed with two species of *Sorghum* and with *Erianthus arundinaceus* at Coimbatore (Venkatraman, 1938).

These grasses are all within the group Andropogoneae according to the accepted classification of Bews (1929). A wider cross would be expected to succeed less readily. In 1936 I crossed several inflorescences of "Vellai" with pollen of *Zea Mays*, and obtained a single seedling, which proved to be a true hybrid (Janaki-Ammal, 1938a). I repeated the cross in 1938 and obtained another seedling, which, however, died early. Dr S. C. Harland tells me that, using a different type of sugar cane, he attempted a similar cross in Trinidad, but without success.

The variety of *Zea Mays* used as male parent was a sweet corn, "Golden Beauty". It was grown for several generations without chance of cross-pollination with other varieties, being the only kind of maize grown in the neighbourhood.

2. Methods

Canes of the male sterile *Saccharum officinarum* "Vellai" which was about to "arrow" were transported from the field and planted horizontally into a pollinating shed. The inflorescence, a large panicle, was supported by bamboo stilts to raise it above the ground. The arrows were

bagged as soon as they emerged from the sheath. When stigmas started to appear they were dusted with pollen of *Zea Mays*. This was continued daily for about ten days. Two arrows under bag were allowed to remain in the same shed as control.

The seeds from the pollinated arrow were grown in the usual way (Barber, 1916) and a single seedling appeared. Seeds from unpollinated arrows failed to germinate.

The obstacle in crossing *Saccharum* with *Zea* seems to lie in the first stage of the operation, viz. in the widely different sugar concentrations required by the germinating pollen. Pollen of *Zea Mays* was tested for germination in different concentrations of sucrose in 1 % agar. *Zea* pollen germinates in concentrations ranging from 10 to 18 % sucrose. This is much below the concentration needed for sugar cane pollen, which demands the narrow range of 23–25 %, presumably the percentage of sugar present in the stigmas.

The seedling in its early stages was of very weak growth, and special treatment with nutrient solutions was used to keep it alive.

Root tips of the two parents and the hybrid were fixed in Allen's Bouin and in La Cour's 2 BD. Roots of *S. officinarum* were pre-treated with ice.

3. CHROMOSOME NUMBERS IN PARENTS AND HYBRID

S. officinarum "Vellai" showed 80 chromosomes, as was found by Dutt & Subba Rao (1933). The length of the chromosomes varied from 3·6 to 1·6 μ. Both primary and secondary constrictions were found, but in no case was I able to detect any chromosome with satellites (Fig. 18 *a*).

Zea Mays "Golden Beauty" had besides the usual 20 chromosomes two *B* chromosomes, which could be distinguished by their deeper staining. The different chromosomes of the haploid complements of maize were recognizable by their sizes, the position of the centromere and the satellite of chromosome VI (Fig. 18 *b*).

The hybrid *Saccharum-Zea* had 52 chromosomes, the sum of the haploid complements of *Saccharum* and *Zea*. The two *B* chromosomes seem to have been transmitted to the hybrid. It was not possible to distinguish all the *Zea* chromosomes in the hybrid, but a single satellite chromosome was seen (Fig. 18 *c*) as well as one of the *B* chromosomes. The *Zea* chromosomes appear to have undergone considerable size reduction in the hybrid, indicating genotypic control of size (Darlington, 1937, p. 55).

4. Morphology of parents and hybrid

The parent plants are too well known to need detailed description. The cane "Vellai"—the name means "white" in Tamil—is very probably the cane Lahaina of Hawaii. It was known as early as 1766 when
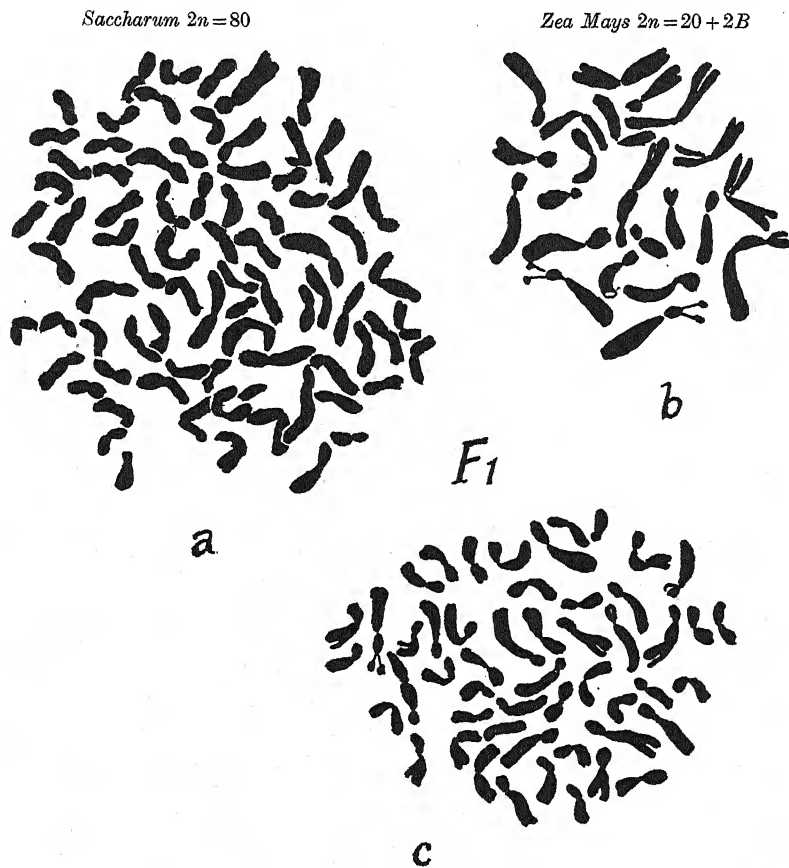

Fig. 18. Root tip metaphase in *a, Saccharum officinarum* Vellai; *b, Zea Mays* Golden Beauty; *c, Saccharum-Zea* hybrid. ×4000.

Bougainville introduced it from Otaheite to Mauritius and Bourbon, now Reunion (Deerr, 1921). In 1791 Captain Bligh brought it from Otaheite to Jamaica (Earle, 1928). It is known as the Otaheite cane, also as the Bourbon or Cana Blanca. It has a thick soft stem, and is still cultivated in many parts of the world as a chewing cane. A full description will be found in Barber (1916) and Earle (1928).

The *Zea* parent "Golden Beauty" is a sweet corn sold in Poona as a type suitable for cultivation in India. Like all maize it varies enormously in size according to soil and cultivation. In the loamy soil of Coimbatore it attained a height of about 5 ft. The ear was 6–7 in. long.

The hybrid *Saccharum-Zea* in the young seedling stage resembled a small *Saccharum*. After a year the plant was barely a foot high; during the second season it put out a number of tillers (Fig. 19). After four years it remains a dwarf bush, recalling in general appearance *Tripsacum dactyloides* as illustrated by Mangelsdorf & Reeves (1939). The growth of the main axis is very much retarded by the vigorous side branches. The
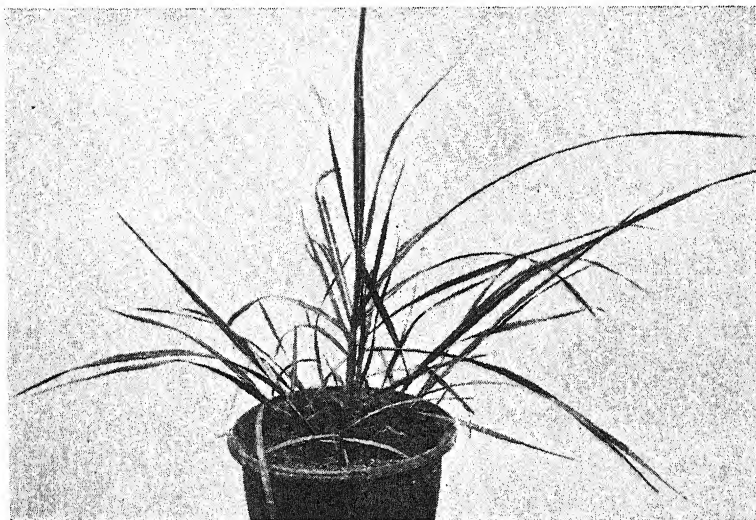

Fig. 19. *Saccharum-Zea* hybrid during second year of growth.

perennial nature of the hybrid has made it possible to propagate it vegetatively. Each tiller looks like a diminutive sugar cane with small *Saccharum*-like stem and leaves and has a ligular process, as found in Vellai but not in *Zea* (Fig. 20). The plant shows no sign of flowering.

The upper surface of the leaf is covered with long silky hairs, similar to but larger than those in *Zea* (Fig. 21). These silky hairs are a feature I have not found in any *Saccharum* except a freak cane "Troeboe" from Java. "Troeboe" is of unknown origin, and I am tempted to consider it as a possible hybrid between *Saccharum* and a member of the Maydeae. The possibility is strengthened by the fact that it does not bear perfect flowers; its inflorescence is aborted and forms a cauliflower-like mass in

the sheath. Incidentally, this malformation seems to have led to the plant's survival in cultivation, as I am told the inflorescence is eaten as a salad by the peoples of the East Indies.

In Table 9 I have tabulated some anatomical and morphological characters of the two parents and the hybrid. The plants have been compared for twenty-four characters, fifteen qualitative and nine quantitative. It will be seen that the hybrid resembles its *Saccharum* parent in ten of the qualitative characters and *Zea* in three. One character is
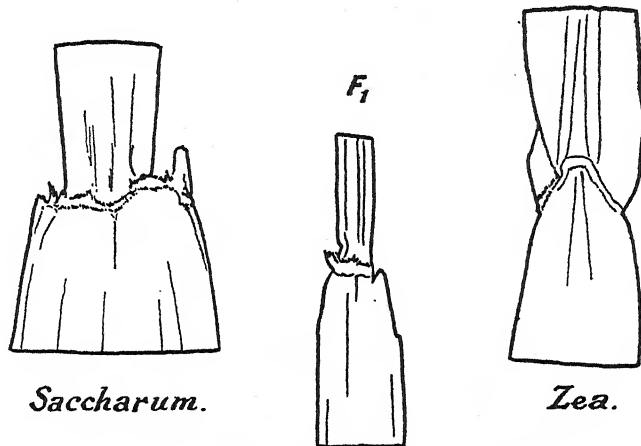

Fig. 20. The ligule in *Saccharum*, *Zea* and the F_1 hybrid.

intermediate and one is new in the hybrid—the depressed shape of the bulliform or motor cells.

In all measurements the hybrid is found to be smaller than either of the parents. This was noticed especially in the size of the cells. Exceptions are the length of the epidermal hairs and the number of vascular bundles in the stem of the hybrid plant. The concentration of vascular bundles (Fig. 22, F_1) can be explained as due to the extreme reduction of the stem and the consequent entry of large numbers of leaf traces into it.

The hybrid was not examined for its sugar content, but from the extreme woodiness of the stem it is unlikely that it will prove to have much sugar.

5. SUMMARY

1. *Saccharum officinarum* "Vellai", $2n=80$, when crossed with *Zea Mays*, $2n=20+2B$, gave two seedlings, one of which survived and was found to have 52 chromosomes.
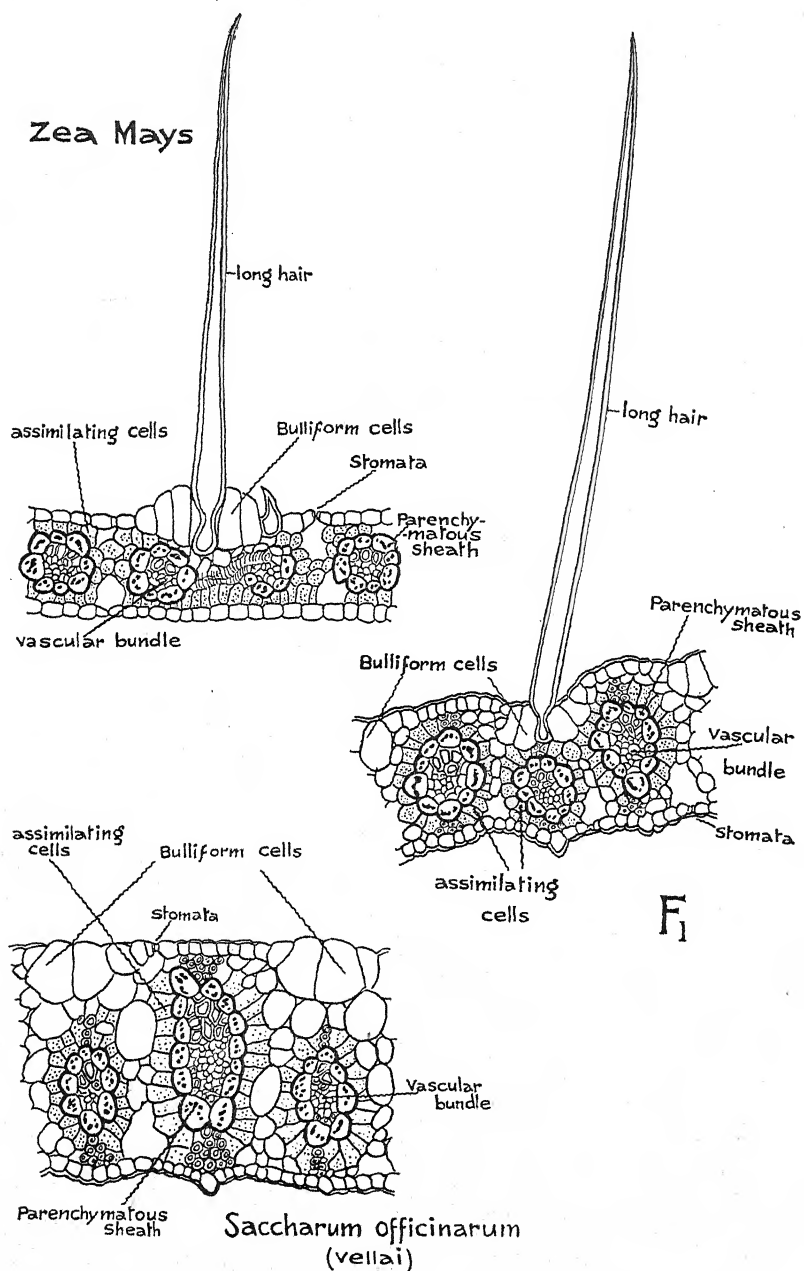
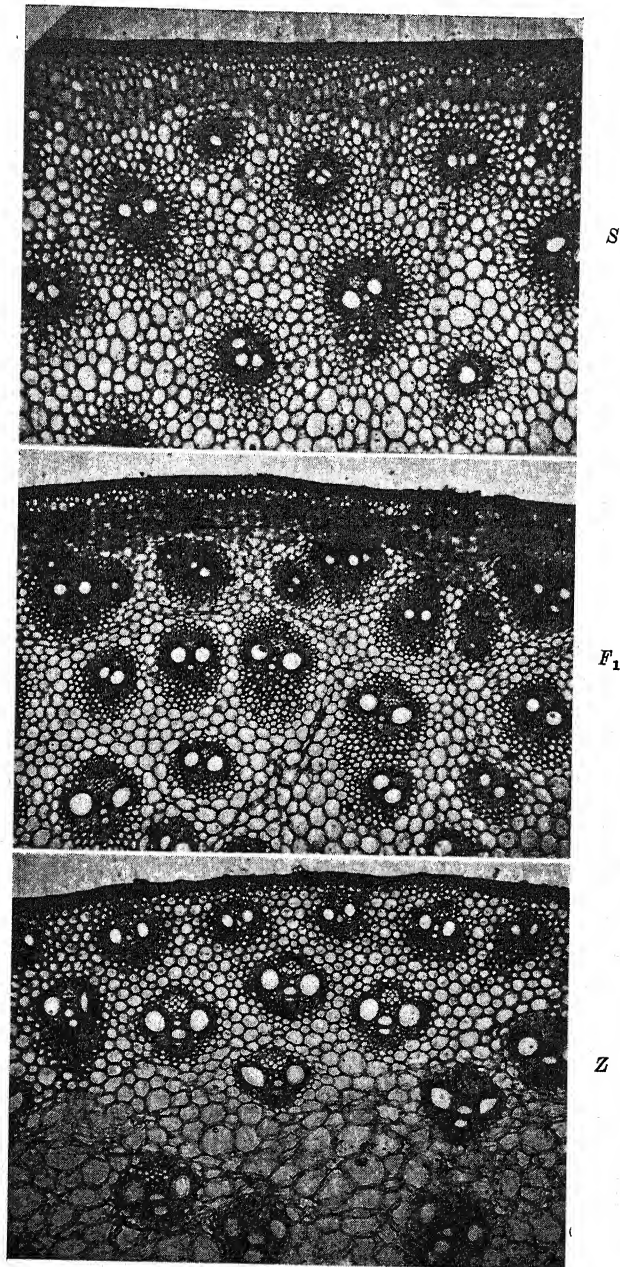Fig. 21. Transverse section of leaf of *Saccharum, Zea* and the F_1 hybrid.

Fig. 22. Transverse section of internode of *Saccharum* (*S*), *Zea* (*Z*) and the F_1 hybrid.

2. The difficulty in making the cross seems to be that the concentration of sugar required by germinating maize pollen is much lower than that found in the *Saccharum* stigma.

3. The hybrid showed some of the detailed characters of each parent, but in general the growth was depressed. In four years the plant has

Table 9. *Comparison of characters of* Saccharum officinarum, Zea Mays *and* F$_1$ *hybrid*

	Saccharum	F$_1$	Zea
Qualitative characters			
1. Habit	Perennial	←———	Annual
2. Root stalks	Present	←———	Absent
3. Root eyes	Present	←——→	Absent
4. Tillering	Many	←———	Few
Leaf			
5. Sheath	Hairy	———→	Glabrous
6. Ligular process	Present	←———	Absent
7. Upper epidermis	Non-hairy	———→	Hairy
8. Lower epidermis	Asperites present	←———	No asperites
9. Vascular bundle	Oval	Intermediate	Round
10. Sclerenchyma in bundle sheath	Present	←———	Absent
11. Bulliform cells	In line with epidermis	Depressed	Raised
12. Shape of bulliform cells	Round	———→	Linear
Stem			
13. Cortex	Present	←———	Absent
14. Distribution of vascular bundles	Diffuse	←———	Peripheral
15. Direction of growth of bundle sheath	Towards stem centre	←———	Equally around bundle
Quantitative characters			
16. Average height (cm.)	600	75	180
17. Stem diameter (cm.)	4	1	2
18. Length of internode (cm.)	9	1·4	18
19. Leaf width (cm.)	6·5	1·5	4·5
20. Leaf length (cm.)	150	36	45
21. Number of bulliform cells	2–3	3–4	3–5
22. Number of vascular bundles per unit area	10	30	20
23. Average length of hairs (mm.)	—	0·75	0·5
24. Diameter of parenchyma cells (mm.)	0·15	0·075	0·125

failed to flower, although it has grown freely and has been propagated from tillers.

4. The vegetative abnormality of the hybrid is attributed to the remoteness of the parents.

5. The hybrid shows a hair character of the Maydeae which is also found in the highly aberrant and sterile cane "Troeboe". "Troeboe" may therefore be of similar hybrid origin.

Table 10. *Recorded intergeneric hybrids of Saccharum*

Clone	2n	Cross reported by	F₁		Chromosome numbers determined by
			Chromosome	Fertility	
I. S. officinarum, 2n = 80					
1. Vellai × *Narenga narenga*	30	Barber, 1916	55	S.*	E.K.J. 1938 (R) ‖ ¶
2. EK 28 × *Erianthus sara*	60	Rumke, 1934	60–70	F.†	Rumke, 1934
3. Vellai × *E. arundinaceus* (as *S. arundinaceum*)	60	Venkatraman, 1935	70	S.?	E.K.J. 1938 (R)
4. Vellai × *Sorghum durra*	20	Venkatraman, 1935	50, 90	N.Fl.‡	E.K.J. 1938 (R)
5. Vellai × *S. halepense*	40	Venkatraman, 1938	60	P.S.§	E.K.J. 1938 (R)
6. Vellai × *Zea Mays*	20 + 2 B	E.K.J. 1938 a	52	N.Fl.	E.K.J. 1938
II. S. spontaneum					
7. Glagah 112 × *Er. ravennae*	20	E.K.J. 1936 (R)	66	F.	E.K.J. (unpublished)
8. Hole's no.1 56 × *Sorghum durra*	20	E.K.J. & Singh, 1936	38	S.	E.K.J. 1938 b
			66	F.	
9. Coimbatore 64 × *Sorghum durra*	20	E.K.J. 1936 (R)	42	P.S.	E.K.J. (unpublished)
10. Gigas 124 × *Sorghum durra*	20	E.K.J. 1936 (R)	—	P.S.	—
11. Gigas 124 × *S. halepense*	40	E.K.J. 1936 (R)	—	P.S.	—
12. Coimbatore 64 × *Bambusa arundinacea*	72	E.K.J. 1939 (R)	68	P.S.	E.K.J. (unpublished)
				1 F₂ seedling	
III. S. officinarum × S. spontaneum and derivatives					
13. POJ 2725 106 × *Sorghum durra*	20	Thomas and Venkatraman, 1930	63–4	P.S.	Singh, 1934
			116–118		
14. POJ 2725 106 × *Sorghum* sp. (as *Holcus Sorghum*)	20	Bourne, 1935	—	P.S.	
15. POJ 2725 106 × *Imperata cylindrica*	20	E.K.J. 1938 (R)	120–134	F.	E.K.J. (unpublished)
16. POJ 2725 106 × *Bambusa arundinacea*	72	Venkatraman, 1937	90	F.?	E.K.J. 1938 (R)
17. Kassoer 136 × *Sorghum durra*	20	E.K.J. 1936 (R)	—	—	—
IV. S. officinarum × S. Barberi					
18. POJ 213 124 × *Bambusa arundinacea*	72	Venkatraman, 1937	96–100	F.	E.K.J. 1938 (R)

* S. = sterile.   † F. = fertile.   ‡ N.Fl. = non-flowering.   § P.S. = pollen sterile.   ‖ E.K.J. = E. K. Janaki-Ammal.
¶ R = *Report of work done under the Scheme for Research on the Genetics of Sugar Cane*, Government of India Press, Simla, 1936 *et seq.*

GENERAL DISCUSSION AND SUMMARY

Table 10 gives a list of the recorded intergeneric hybrids of *Saccharum*, including those described in the present paper.

It appears that in *Saccharum* high polyploidy has removed all obstacles to hybridization with other groups of Gramineae except those that depend upon the simply ascertainable conditions of pollen germination. The mode of action of high polyploidy is fully displayed in the versatile method of reproduction of the "nobilized" hybrids of *S. officinarum* and *S. spontaneum*, which in this respect resemble polyploids in *Poa* and *Rubus*. They are capable of producing from apparent hybridization with diploid species of other genera true diploid crosses, true triploid crosses and diploids and triploids which are not crosses at all. The fertility of the progeny depends not so much on the remoteness of the cross as on the internal pairability of the chromosomes derived from the polyploid parent, in other words on their capacity for autosyndesis. Systematic study of these properties will enable us in the future to recombine the materials of plant improvement on a scale that has not hitherto been realized.

ACKNOWLEDGEMENT

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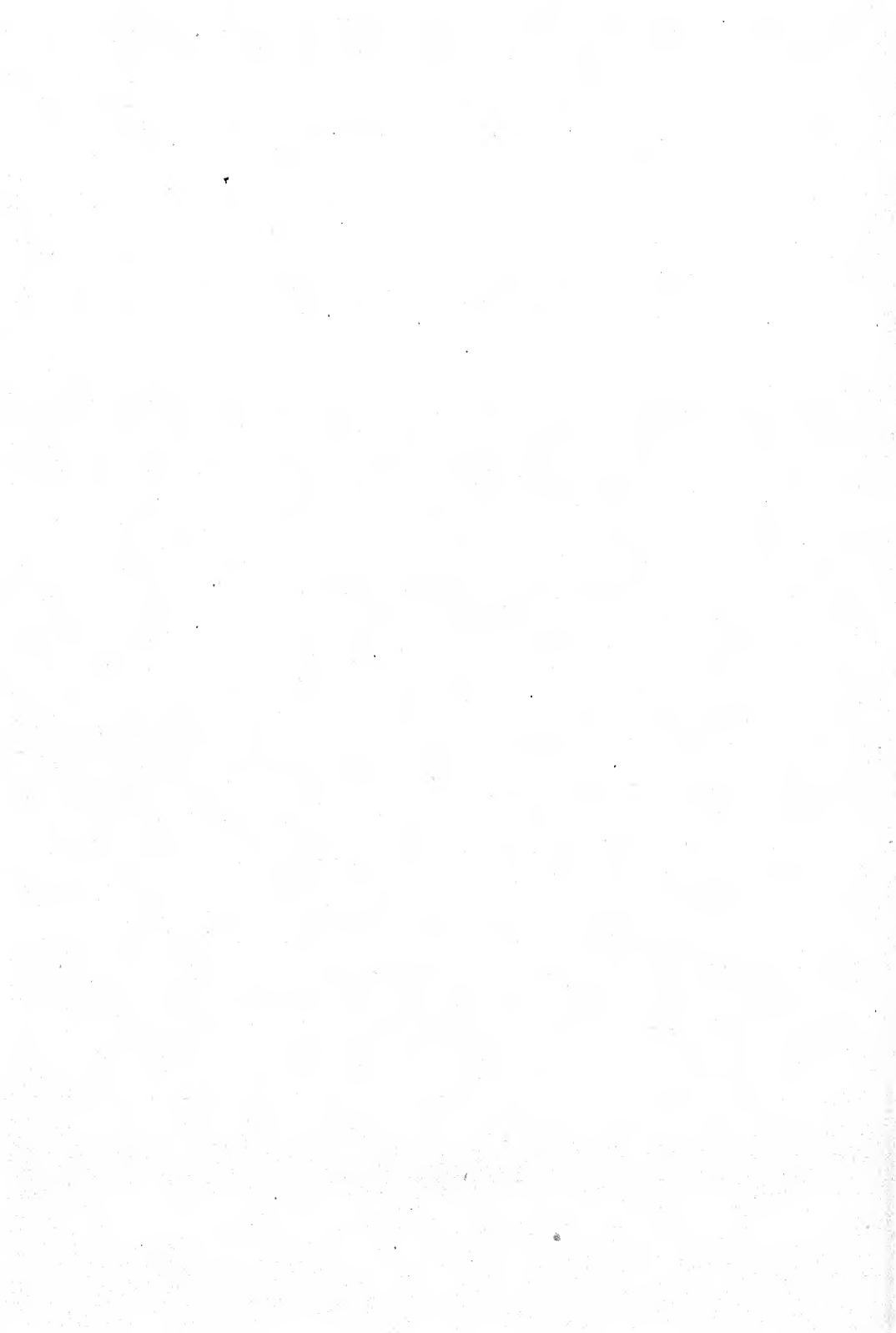
THE EFFECT OF SEX ON THE SPONTANEOUS MUTATION RATE IN *DROSOPHILA MELANOGASTER*

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INTRODUCTION

In experiments designed to test the influence of carcinogenic substances on the mutation rate in *Drosophila melanogaster* (1940), pronounced differences were observed between the rates with which sex-linked lethals arose spontaneously in male and female germ cells. The flies used for testing the mutation rate were F_1's from crosses between females of a $sc^8 w^a bb$ stock and males of a $sc v \delta 49 \, od \, ca$ stock. When only the lethals arising in the $sc^8 w^a bb$ chromosome are considered the figures are: no lethal in 846 chromosomes derived from females, and twenty-seven lethals (from twenty-four different individuals) in 3771 chromosomes derived from males. These results had been guarded against any error due to the occurrence of non-disjunction by the scheme of crossing used. Results pointing in the same direction had been reported by Muller & Altenburg as far back as 1919, but as the differences then observed were not statistically significant, and as significant data were difficult to obtain with the low natural mutation rate soon afterwards found in the stocks used, these indications were not followed up. The present differences, however, were so striking that further investigations into this problem of fundamental importance appeared promising.

EXPERIMENT I

Following the above preliminary results, the first test was carried out with the $sc^8 w^a bb$ stock which had been used as one of the parental stocks in the experiments on carcinogenic substances. Males and females to be tested for the occurrence of sex-linked lethals in their germ cells were taken from the same stock bottles. The males were tested by the usual *ClB* method, the females by means of the following scheme of crosses:

$$P_1 \, \female \, \frac{sc^8 w^a bb}{sc^8 w^a bb} \times \male \, sc v \delta 49 \, od \, ca \text{ (pair-matings, 23 pairs);}$$

$$F_1 \, \female \, \frac{sc^8 w^a bb}{sc v \delta 49 \, od \, ca} \times \text{brother } sc^8 w^a bb \text{ (pair-matings, 30–60 pairs from}$$

each $P_1 \, \female$).

In the absence of a sex-linked lethal the F_2 males consist of two types which are readily distinguished through the glass wall of the culture vial. If a lethal arises in the germ track of a P_1 female, one of her daughters fails to produce w^a sons, the production of cross-overs by the F_1 being prevented by the presence of different inversions in the two X-chromosomes. By mating the P_1 in pairs any lethal already present in a P_1 female could be detected by the low sex ratio of her offspring, and such females were excluded from the test. Likewise excluded were P_1 females which produced non-disjunctional $sc v \delta 49 od ca$ sons, because the appearance by secondary non-disjunction of w^a F_2 males might mask the presence of a lethal on the w^a chromosome. There still remain the possibilities of primary non-disjunction in the F_1, which in the presence of several inversions cannot be neglected, and of an extra Y introduced from the P_1 male causing secondary non-disjunction in the F_1. The precaution against these sources of error in experiment I was not to classify any F_2 progeny as lethal-free unless at least three w^a males were found on superficial inspection through the glass of the vial, and in doubtful cases to rear an F_3. This seems sufficient to exclude cases of primary non-disjunction, but some cases of secondary non-disjunction due to the presence of an extra Y in the P_1 male may have remained undetected and create a source of error which is not altogether negligible.

The results were as follows: no lethal in 843 chromosomes derived from females; five lethals and one semilethal (one male among more than fifty females) in 538 chromosomes derived from males.

EXPERIMENTS II AND III

In order to eliminate differences of genotype—apart from those necessarily existing between the sexes—the following tests were carried out with males and females from Florida wild-type stocks made isogenic by Singh through a sequence of crosses described in his thesis (1940). Two of these stocks were used: "Florida 4" and "Florida 5" (*Fo* 4 and *Fo* 5). As in each of these stocks by far the greater part of the major chromosomes of every individual is derived from one and the same ancestral haploid set, these flies constitute a nearly homogeneous material in respect of genotype—barring, of course, new mutations which may have arisen between the time the stocks were completed and the beginning of our experiments. At the same time, environmental differences between the flies under test were reduced to a minimum by rearing them under controlled and as nearly as possible identical conditions of food, temperature, and moisture, by taking P_1 males and females from the same

bottles in approximately the same numbers, and by randomizing the unavoidable individual differences between P_1 individuals through the use of a fairly high number of P_1 couples in each series. Males were again tested by the ClB method, females were tested by the following crosses:

$$\text{``}P_0\text{''}^1 \,\female\, \frac{Fo}{Fo} \times \male\, Fo \text{ (controlled and identical conditions)};$$

$$P_1 \,\female\, \frac{Fo}{Fo} \times \male\, sc^{S1}Lw^a sc^4R \text{ (23 pairs in experiment II, 37 pairs in}$$

experiment III);

$$F_1 \,\female\, \frac{Fo}{sc^{S1}w^a sc^4} \times \text{brother } Fo.$$

A lethal in the Fo chromosome becomes apparent by the absence of wild-type males in F_2. As before, precaution was taken against lethals present from the start, and against secondary non-disjunction due to a Y-chromosome introduced from a P_1 female. Moreover, in each batch of F_1 females derived from a P_1 pair, a number of females were mated as virgins to yw^aB males. If a Y-chromosome had been handed on from the father, some of these females would be expected to produce sons of paternal type, and in this case the whole batch was discarded. By accepting as lethal-free only those F_2 progenies in which at least three wild-type males were observed through the glass of the vial and by subjecting the doubtful cultures to further breeding tests, precaution was taken against occurrences both of primary non-disjunction in cells of the F_1 $\female$ and of double crossing-over between the two X-chromosomes of the F_1 female. The results were as follows:

Experiment II. No lethal in 815 chromosomes derived from Fo 5 females. Nine lethals (from six different males) in 841 chromosomes derived from Fo 5 males.

Experiment III. No lethal in 796 chromosomes derived from Fo 4 females. One lethal and one semilethal (three males among more than seventy females) in 790 chromosomes derived from Fo 4 males.

EXPERIMENT IV

The data of experiment III, though not disproving the earlier results, yet do not confirm them. It was therefore deemed desirable to test the question again on a larger scale. One more experiment was carried out, using Fo 5. The technique was the same as before except for four alterations: (1) Special care was taken to test germ cells of young individuals

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[1] "P_0" is used to designate the generation preceding that of the flies ("P_1") whose mutation frequency was tested.

only, by mating the P_1 flies a few days after collection, keeping them on syrup food between collection and mating, and removing them from the vials after 3–4 days. (2) $sc^{S1}Lw^a In\text{-}S sc^8 R$ males that had been made up for such purposes by Muller were used for the P_1 instead of the rather inviable $sc^{S1}w^a sc^4$ males. The presence of inversion S in the middle of the X-chromosome (Muller, 1935) renders the suppression of cross-overs complete. (3) In the later part of the experiments, the F_1 females were mated to $y^2 sc^8 w^a B$ males. Though the females were not virgins, a sufficient number of B daughters were usually produced to allow an easy decision whether the absence of w^a males in certain F_2 progenies was due to a lethal in the $sc^8 w^a In\text{-}S sc^{S1}$ chromosome or to the mother (F_1) having been a homozygous wild-type ♀ derived by primary non-disjunction in the P_1 female or by her non-virginity; thus simultaneous observation of lethals in both chromosomes could be carried out with only a little more labour. (4) For detecting an extra Y in the P_1 males each male was tested by mating it to a virgin female carrying $bw^{v4} Bl L^2$. The presence of an extra Y is easily discovered in the offspring by the appearance of a number of non-Bl non-L^2 flies in which the mottling of the eye has been suppressed. All daughters of P_1 males with extra Y's were excluded from the test.

The results of experiment IV were as follows: three lethals (two of them from the same female) and one semilethal (two wild-type males) in 2744 chromosomes derived from Fo 5 females; fifteen lethals and one semilethal (five males among over fifty females) in 2691 chromosomes derived from Fo males. In addition, twelve lethals (from eight different P_1 males) were found among the 2744 $sc^{S1}L In\text{-}S w^a sc^8 R$ paternally derived chromosomes in the series in which the maternally derived Fo chromosomes were being tested. This latter finding may be taken as to some degree confirmatory of the relatively high mutability of the X-chromosome in the male, although of course the flies supplying this w^a-containing chromosome were genetically different from those of Fo 5.

When the data, as tabulated in Table 1, are pooled according to the method developed by Muller (1940)—disregarding the semilethals, and in the male series counting as separate only mutations which arose in different males—the difference in the percentage of sex-linked lethals turns out to be 0·48 % with a standard error of 0·11 %. As the difference is 4·4 times its standard error the result is statistically well secured. Analysis of the data gained in this experiment showed that the apparent discrepancy of the results gained in experiment III from the rest was in all probability only a result of "accidental" circumstances: in experi-

ment IV, also, there occurred one run of over 600 F_2 families without a single lethal.

Table 1. *Summary of experiments I–IV*

		Chromosome derived from female				Chromosome derived from male			
No. of experiment	Chromosome tested	No. of fertile F_1 cultures	No. of lethals (in brackets: semilethals)	From how many different ♀♀	Percentage of lethals	No. of fertile F_1 cultures	No. of lethals (in brackets: semilethals)	From how many different ♂♂	Percentage of lethals
I	$sc^8 w^a bb$	843	0	—	0	538	5 (+1)	6	0·93
II	Fo 5	815	0	—	0	841	9	6	1·07
III	Fo 4	796	0	—	0	790	1 (+1)	2	0·13
IV	Fo 5	2744	3 (+1)	3	0·15	2691	15 (+1)	16	0·56

DISCUSSION

The data presented above appear to establish a difference in the rate at which sex-linked lethals arise spontaneously in the sexes, the males having the higher mutation rate. From what we know about the different types of mutation, there is no reason to suspect that this sex difference should not extend to viable and autosomal gene mutations as well. As to its causes, only assumptions can be put forward as yet. If subsequently it should become possible to decide between them experimentally, this might bring us one step nearer the truth about the origin of natural mutations.

In their qualitative gene content, males and females of an isogenic stock differ only by the presence of the Y-chromosome in the former. It does not seem very likely that the Y-chromosome should influence the occurrence of mutations. Females carrying a Y-chromosome or a portion of it might be used to test this possibility.

When the mutations for which we test are sex-linked lethals, the possible occurrence of germinal selection in the male but not in the female has to be taken into account. Its effect would be to reduce the number of observable lethals in the male. If, therefore, it had occurred in the present experiments to any considerable degree, the observed difference between males and females would assume even more significance.

A possible difference between the sexes which might be considered as underlying the observed difference in mutation rate is one in respect of the number of cell divisions intervening between the fertilized egg that is to develop into the P_1 and that of the next generation (F_1), in which the mutant gene is found to have been present. If this number were considerably higher in the male, and if mutation occurred exclusively or mainly during the process of reduplication of the genes (a possibility

tentatively suggested by Muller, 1928, and apparently supported by results of Olenov, 1939, and of Singh, 1940), a superiority of the male in respect of mutation rate would be expected. Both assumptions, however, are as yet unproved. Muller's suggestion would find support if it could be shown that correlated with the higher frequency of mutants in the Drosophila sperm as compared with the egg was a markedly greater number of mitoses during its life history. Unfortunately, the proof for this is not easy to adduce, though at first sight one would suppose that the larger number of spermatozoa would require a larger number of preceding divisions. To arrive at a rough idea of the number of mitoses between fertilized egg and mature reproductive cell in either sex, the following calculations can be made, taking the female first.

According to Huettner (1923), the polar cells are differentiated from the blastoderm cells at the 256 nuclei stage, i.e. after eight previous divisions. There are five to eleven of them, and they form an average of fifty egg strings (ovarioles) in the mature female (Donald & Lamy, 1937). To obtain fifty initial cells for the fifty egg strings from eight to ten pole cells two to three mitoses are required. The total output in eggs of a *D. melanogaster* female averages about 1000, i.e. about twenty eggs per ovariole. Assuming that twenty oögonia are formed in the end filament as forerunners of the twenty eggs to be produced, and that these twenty oögonia are formed by simple dichotomous division, the numbers of cells after each subsequent division proceeding as the powers of two, four to five oögonial divisions have to be postulated. Almost certainly this figure is too low: if oögenesis followed this system no cells would be left in store at all. One division at least has to be set aside for the purpose of providing a store. Possibly there is considerably more storing. Also, there is no reason to assume that oögonial division always or mostly follows a dichotomous scheme. Certain mitoses may result in two cells, one of which only would go on dividing, the other being kept in store (or possibly becoming non-germinal). The extreme case of this type would be a division scheme in which one apical cell gives off one oögonial cell at a time, all oögonial cells being direct progeny of this apical cell. If, then, the oögonia developed directly into the egg, the first egg to be formed would require one oögonial division, the second two, etc. Twenty divisions would precede the formation of the twentieth egg, and ten divisions would be the average for all eggs formed during the lifetime of the fly. For the first eggs, however, which alone were used in experiment IV, the average would be much lower, perhaps two or three. If we allow each oögonium two more divisions before reaching the oöcyte stage the figure is raised to

four or five, i.e. the same as assumed above for a purely dichotomous mode of oögenesis. Next come four divisions producing the fifteen nurse cells and the egg proper, and finally the two oöcyte divisions. Adding up, we arrive at an estimate of $8+3+5+4+2=22$ mitoses preceding the formation of the mature egg.

In the male-forming egg development up to the formation of the polar cells is the same as in the female-forming egg, i.e. eight initial mitoses have to postulated. The five to eleven polar cells thus formed produce the two testes, which together, according to Kaufmann (oral report from Dr Koller), contain 8000–10,000 completed spermatozoa in the newly hatched male. In order to produce 10,000 spermatozoa from, say, ten initial cells by pure dichotomy, ten divisions (including the two spermatocyte divisions) are required. With an exclusively apical cell scheme of division, one primary cell in each testis would have to give off the 1000–1250 primary spermatocytes necessary to produce 4000–5000 spermatozoa. The number of mitoses preceding the primary spermatocytes would thus range from one for the first to at least 1000 for the last, with an average at 500–625. The spermatocytes then undergo two more divisions. Whereas the apical division scheme allows of a continuous formation of spermatogonia for the subsequent production of spermatocytes, the dichotomous scheme requires some previous storing (say four to five divisions in analogy to the estimate for the female). Adding up, we arrive at a minimum of $8+10+4=22$, and a maximum of $8+500+2$ $=510$ or more mitoses preceding the formation of the sperm in the newly hatched male.

It will be seen that the minimum estimates do not differ for the two sexes. The maximum estimates, on the other hand, differ considerably. It is, however, almost certain that the pure apical scheme is not realized in spermatogenesis. Not only do the results gained by Harris (1929) provide evidence of at least two apical cells in each testis, but also cytological evidence on mitoses in the testes and on the time required is in contradiction to rigid apical proliferation. Most probably, actual spermatogenesis follows a system combining both modes of division. It can be seen that figures to fit any ratio between the two sexes could easily be made up by supposing a suitable intermediate between the two extreme schemes of gametogenesis. However, these remain mere speculations until independent information concerning gametogenesis has been gained.

Harris (1929), arguing from the fact that a mutation produced by X-rays 2–3 weeks previous to mating occurs in one-quarter of the sperm,

comes to the conclusion that "the proliferation of germ cells in the testis probably occurs through a system of one or a very few indefinitely reproducing cells functioning like apical cells". His data are, in fact, reconcilable with any other system of division as long as it is assumed that the sperm used in the late mating goes back to two spermatogonial cells present at the time of raying, and only if the situation as found by Harris after a definite time interval between raying and mating were true in general would it imply the existence of apical cells. Experiments of the same kind as those carried out by Harris, with raying at different ages, and checking on group formation of lethals at intervals of a few days, might, as Muller suggests, help to narrow down the scope of possibilities. Before more evidence on the method of gametogenesis is available, all that can be said in respect of its bearing on the sex difference in mutation rate is that it may conceivably be explained by a corresponding difference in the number of mitoses (including gene reduplications) during gametogenesis.

If we discard this explanation as unfounded, there still remains the possibility that the higher mutation rate in the male is an effect of some other physiological difference between the sexes. It is known for example that the catabolic processes differ between the sexes in many animals, the males having the higher catabolic rate. The hypothesis that metabolic processes should be able to influence mutation rate does not appear too fantastic in view of the influence on mutation rate found to be exercised certainly by temperature (Muller & Altenburg, 1919; Muller, 1928; Plough & Ives, 1932, 1935; Promptov, 1934; Timoféeff-Ressovsky, 1935; Buchmann & Timoféeff-Ressovsky, 1935, 1936; Zuitin, 1937, 1938 *a, b*), and possibly by certain chemicals (Sacharov, 1932, 1933, 1935, 1936, 1938; Lobashov & Smirnov, 1934; Lobashov, 1935; Magrzhikovskaja, 1936, 1938), and by nutrition (Döring, 1937; Stubbe & Döring, 1938; Olenov, 1939). Higher rates of oxidation might influence the mutation rate through direct chemical effects or indirectly through influencing the nature of the medium in which the nuclei exist. If this explanation of the observed differences in germinal mutation rate were true, one would expect to find a corresponding difference in respect of somatic mutations. A higher rate of somatic mutations in the male could not be explained by a greater number of preceding mitoses; an influence of the Y-chromosome might be regarded as responsible, but could easily be tested in XXY females. Another test of the explanation by such differences in metabolism would consist in direct studies of the influence of altered metabolic rates on the occurrence of mutations. Studies of this kind are now in progress at this institute.

Incidentally our data also provide new evidence of the considerable amount of fluctuation of unkown origin in mutation rate already commented upon by others (cf. Muller, 1928), and it is notable in our work that this applies even to material very strictly controlled for genetical and environmental uniformity. Observations like this should serve still further to caution investigators working on spontaneous mutation rates or using them for control data. Reliable figures for spontaneous mutation rate can only be expected by using devices for maintaining such uniformity, by randomizing the remaining variations through the use of a fairly large number of parents, by taking precautions against sources of error through non-disjunction, crossing-over and the like and, above all, by working with sufficiently large numbers.

SUMMARY

The spontaneous mutation rate in the two sexes was studied in flies from various stocks, mainly isogenic wild-type, reared under controlled and identical conditions. It was found to be markedly higher in the male, the difference being statistically significant. Fluctuations were considerable, even within the same experiment, and point to the necessity for strictest control of all conditions when gaining data on spontaneous mutations. Possible explanations for the observed results are discussed, but without further evidence along other lines no decision between them appears possible.

ACKNOWLEDGEMENTS

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"PIGTAIL," A HEREDITARY TAIL ABNORMALITY IN THE HOUSE MOUSE, *MUS MUSCULUS*

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(With Plates 7 and 8 and Two Text-figures)

AMONG the mice supplied by a commercial breeding farm, there occurs occasionally a tail abnormality which, as it appeared to be of a hereditary nature, was subjected to genetical studies. It was intended to supplement these by histological and embryological investigations and to clear up doubtful genetical points by further breeding tests. Since, however, present conditions do not allow of further research, the data gained so far will be presented below without much attempt at interpretation, which, at the present stage of the investigation, must needs be very hypothetical.

DESCRIPTION

The abnormality in question has been called "pigtail", for in its more pronounced degrees of manifestation, the tail forms a complete curl similar in appearance to the curled tail of a pig. The pigtail condition as observed by external inspection, without the aid of X-rays or sections, varies from a slightly perceptible thickening at some point in the tail to complete single or even double curls (Text-fig. 1). Intermediate stages are single or multiple kinks varying from obtuse to right angles, with more or less pronounced bony thickenings at the bending points. The direction of the curls may be right-handed or left-handed. In the case of multiple kinks the direction may remain the same from one kink to the next, or it may change and thus produce a zigzag pattern. When a strong kink or curl occurs at the root of the tail the position of the sacrum and the pelvis bones may be affected so as to become markedly asymmetrical when seen from above. The lowest grade of pigtail may become manifest only some weeks after birth. Thus the various degrees of expressivity of pigtail arrange themselves into a series, the lowest member of which is just recognizable on inspection of the live animal and remains unidentified should the animal die in infancy. A continuation below this level would lead to a condition which is no longer manifest to the unaided observer, though indications of it might be discovered by

special techniques, such as histological preparations. In other words, variable expressivity will be combined with incomplete penetration (Timoféeff-Ressovsky, 1934). As will be seen below, the actual breeding results allow of a reasonable interpretation only when it is postulated that a considerable proportion of genetically pigtail animals overlap normal.

It was thought possible that X-ray photographs might reveal bone abnormalities in all or most of these overlaps. A great number of radiograms gave not the least support to this assumption. Whereas the
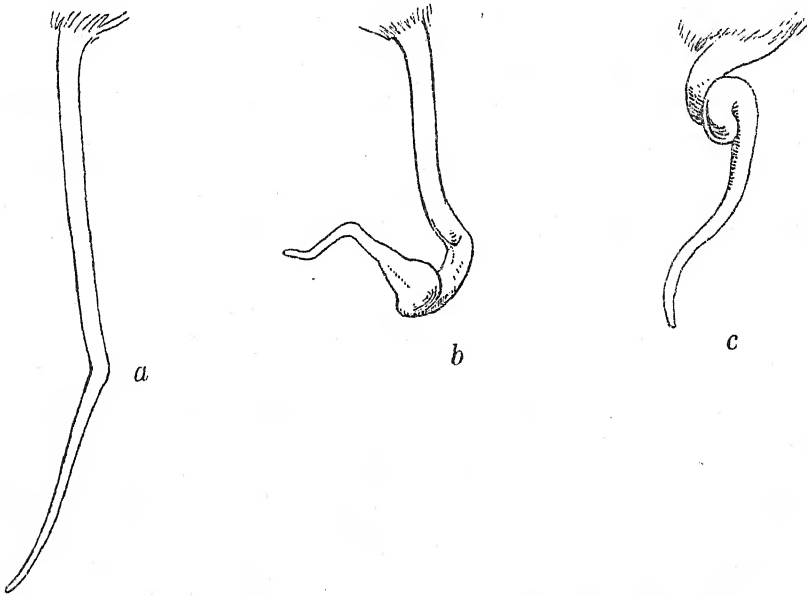
Text-fig. 1. Various degrees of expression.

manifest pigtail condition exhibits more or less marked abnormalities of the shape of the tail vertebrae (Pl. 7, fig. 1), combined with ankylosis and fusion of vertebrae, the skeleton of the overlaps (Pl. 8, fig. 3) resembles that of the controls (Pl. 8, fig. 4) in every detail. It should be pointed out, however, that the inspection of the X-ray photographs was a purely qualitative one, no measurements of vertebrae being taken, since there was not much reason to suppose that a quantitative study would reveal differences between controls and phenotypically normal, genotypically pigtail individuals.

Tail skeletons freed from the adhering tissues corroborated the evidence from X-ray photographs: the overlaps were indistinguishable

from the controls, while the manifest pigtails exhibited abnormally shaped and fused vertebrae.

Bone abnormalities in parts of the skeleton, other than the tail, were not observed; but in later generations a number of young were born with a spina bifida aperta (Pl. 7, fig. 2).

Genetical results

Table 1 summarizes the results gained by breeding from the various types within the stock and by outcrossing and backcrossing to pigtail. Classification for abnormality was done on the basis of naked eye

Table 1. *Results of experimental matings*

| | Crosses within pigtail stock | | | | | Outcrosses | | | | |
	I (a) Both parents manifest pigtails	II (b) Only father manifest pigtail	III (c) Only mother manifest pigtail	IV (b) and (c) combined	V Both parents phenotypically normal	I F_1, father from pigtail stock	II F_1, mother from pigtail stock	III F_1, combined	IV F_2	V Backcross F_1 to pigtail stock
No. of litters	36	10	9	19	21	20	9	29	—	—
No. of young	219	66	51	117	143	173	61	234	311	142
Litter-size	6·1	6·6	5·7	6·2	6·8	8·7	6·8	—	—	—
No. of abnormal young	52	14	10	24	24	0	0	0	5	6
Proportion of manifest pigtails, 1 in	4·2	4·7	5·1	4·9	6	—	—	—	62·2	23·7
No. of litters with spina bifida	4	3	1	4	2	0	0	0	0	0
No. of young with spina bifida	4	6	1	7	2	0	0	0	0	0

inspection and palpation of the tail, counting as abnormal each individual which revealed even a slight abnormality to these methods. As mentioned above, the lowest degree of pigtail may become manifest only at several weeks of age. The inaccuracy introduced by this practice is considerable: in a series of litters which were carefully observed from birth to weaning, eight cases of delayed manifestation occurred among 56 young which at birth were classified as normal and survived the weaning age of 21 days. Therefore, all litters for computation of segregation ratios were kept for 21 days and classified twice, once immediately after birth, a second time at 21 days of age. Even this precaution is, of course, not sufficient to avoid completely the inaccuracy due to delayed manifestation. Deaths at birth or during the suckling period may remove infant mice which were on the way to manifesting a low grade of pigtail. However, as

there is no indication of a selective pre-natal or post-natal mortality of the pigtail young—apart from the spinae bifidae, none of which survived weaning age—it may be assumed that losses due to these causes are evenly distributed among normal and abnormal young.

Outcrosses. Twenty litters by manifest pigtails mated to unrelated inbred agouti, albino, or lemon (**bbppc^{ch} c^{ch}**) mice contained 173 young without a single pigtail. Since at this stage of the experiment, litters were often discarded before weaning, a second series of outcrosses was made, in which the young were kept for 21 days. No pigtail was found among sixty-one young from nine litters. It therefore seems safe to conclude that the genetical basis of pigtail is either a recessive gene or a combination of genes, one at least of which is recessive. In Table 1, the data for the outcross F_1 are grouped according to which parent was pigtail. It will be seen that the pigtail ♀♀ in this series were definitely less fertile than the not-pigtailed ones. The difference in litter size is $1·9 + 0·84$ which is $2·3$ times its standard error.

Inbreeding. Manifest pigtails, when mated together, produce pigtails as well as normals. The proportion of pigtail young in litters from such matings is a little less than 25%. The normal young from these litters when tested adequately, always produced pigtails in a somewhat lower, but not significantly different percentage (columns II–V in Table 1). This fact suggests that the manifest pigtails are not genetically different from the normal litter-mates. From Table 1, it would appear as though the fertility of the manifest pigtail ♀♀ was lower than that of the phenotypically normal ♀♀ (compare columns II and V with I and III), but this difference is not significant on these data. The breeding results do not point to the action of genetic modifiers as responsible for the different degrees of expressivity and penetration, for good pigtails were obtained from normal parents and vice versa. Moreover, selection for high penetration was not successful, but as the highest number of generations in any selected line is only four, this negative evidence is only of slight value. If the degree of penetration depended on the cumulative action of several recessive modifiers for which the strain would become more homozygous through inbreeding, it would be expected that inbreeding *per se* would tend to alter the percentage of manifestation. The coefficient of inbreeding (computed after Wright 1922) without selection varied from 0 to 0·5. Within this range no significant correlation between penetration and degree of inbreeding could be established. The coefficient of correlation, though positive, namely, $+0·191$, is not significant for 74 degrees of freedom.

Among extraneous conditions, the position of the foetus *in utero*, the size of the litter and the physiological state of the mother might be of importance for the development of the abnormality. Dissections of six pregnant manifest pigtail females mated to similar ♂♂ gave no indication of a relationship between visible abnormality and position in the uterus at a stage when the abnormality is already visible in the foetus (16th to 17th day). The age of the mother varied between 2 and 8 months, and within these rather narrow limits no correlation between age of mother and penetration could be established.

As seen from Text-fig. 2, there was a tendency for penetration to decrease with the size of the litter. This observation was borne out by
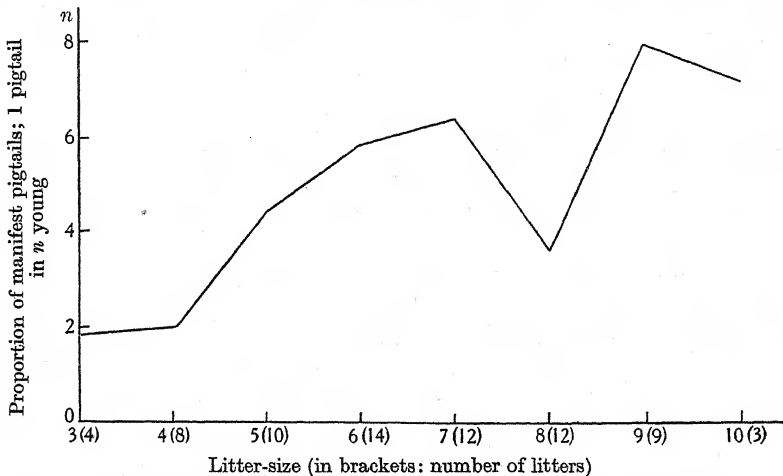
Text-fig. 2. Relation between litter-size and manifestation in genetically pigtail litters.

computation of the coefficient of correlation between litter-size and penetration in litters from two pigtail parents. The coefficient, though small, is significantly negative, namely, 0·23 for 76 degrees of freedom, with the 5% point at 0·217 and the 1% point at 0·283. This fact seems to suggest an influence of uterine conditions on the development of the abnormality.

The last two rows of Table 1 show that young with a spina bifida aperta occurred in litters from all types of mating within the pigtail stock. There appears to be a relationship between the degree of inbreeding and the occurrence of spina bifida aperta, for the average inbreeding coefficient (as calculated from the last four generations) for the ten litters with spina bifida was 0·31 as compared with the average for all matings within the stock of 0·20. The difference is 0·11 ± 0·019. Pene-

SUMMARY

A skeletal abnormality, termed pigtail, in the house mouse is described which phenotypically resembles flexed tail. In a certain small proportion of litters, one or more young with spina bifida aperta occurred, especially in the more highly inbred litters. Genetically, pigtail is a recessive character, caused either by two complementary factors, or—more probably—by one main gene interacting with a number of modifiers which affect the penetration. Penetration is never 100%. In genetically pigtail litters, it varies round about 20%. It is not noticeably dependent on age of mother, degree of expression in the parents, degree of inbreeding or selection; but there exists a significant, though slight, negative correlation between litter-size and percentage of manifestation in homozygous litters, suggesting some intra-uterine, non-genetical influence on the development of the abnormality.

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EXPLANATION OF PLATES 7 AND 8

PLATE 7

Fig. 1. X-ray photograph of the tail of an adult manifest pigtail.

Fig. 2. Spina bifida aperta in new-born young.

PLATE 8

Fig. 3. Skeleton of normal control ♀.

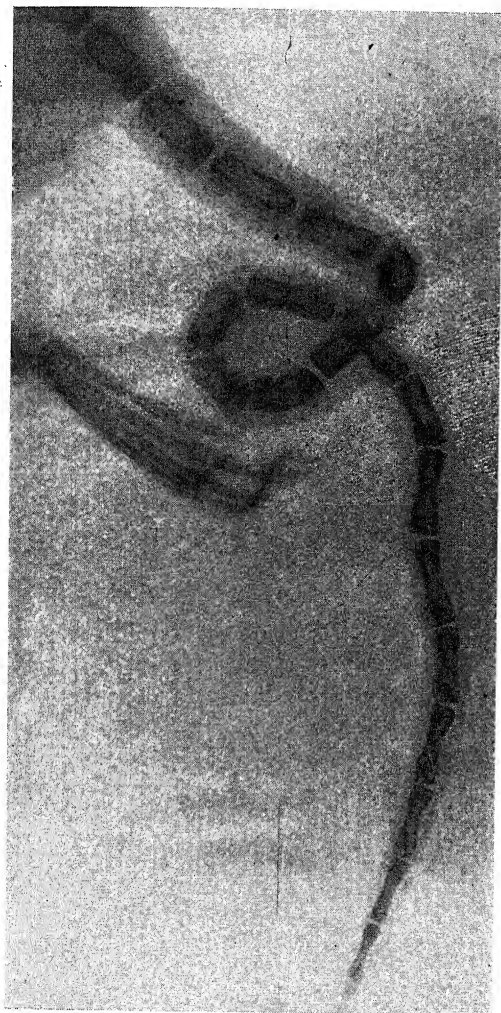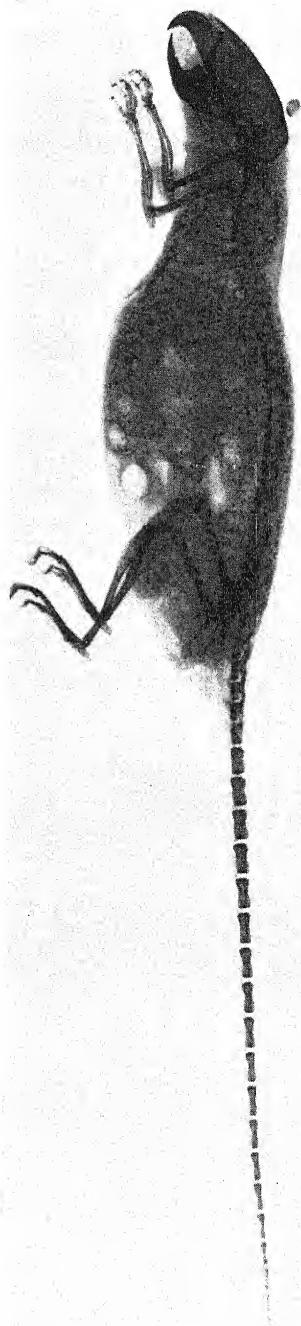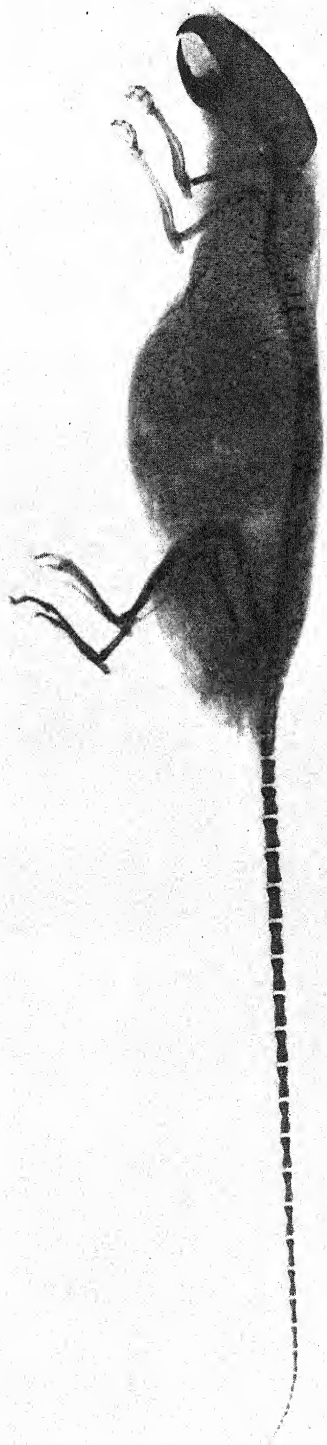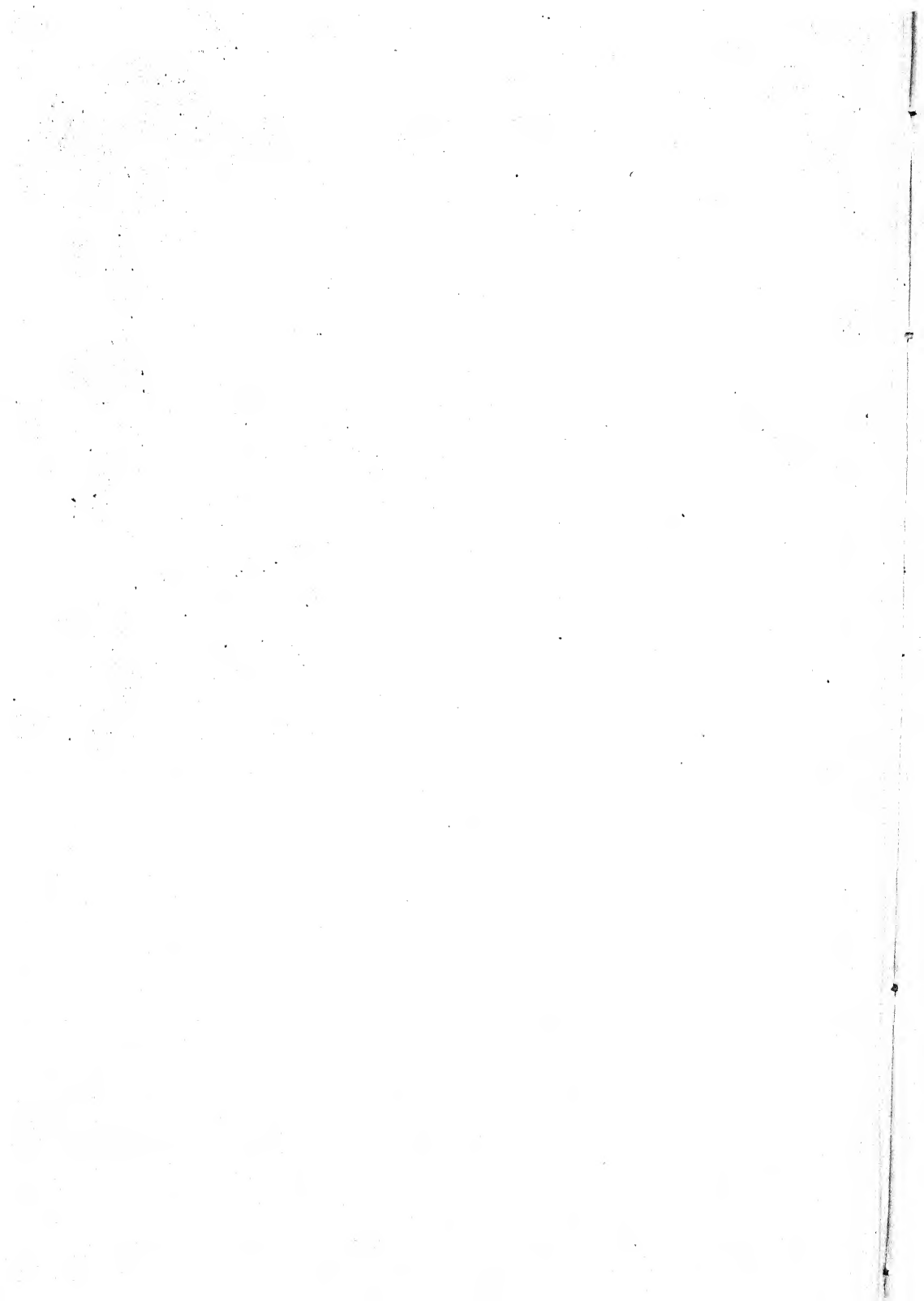Fig. 4. Skeleton of phenotypically normal, genetically pigtail ♀.

Fig. 1.

Fig. 2.

THE ACTIVITY OF INERT· CHROMOSOMES IN *ZEA MAYS*

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(With Plate 9 and Eleven Text-figures)

CONTENTS

1. KINDS OF CHROMOSOMES

IN all sexually reproducing plants and animals there are a certain number of chromosomes whose regular division at mitosis and segregation at meiosis is a condition of regular growth and reproduction. Such chromosomes are therefore constant in number within species or related groups of uniform character. Now these chromosomes, being necessary, are assumed to be active, and rightly so, for any chromosome which is not doing something in the stock that is carrying it will sooner or later be lost by that stock. It will succumb to the hazards of mitosis.

Other chromosomes of a second kind are those which are variable in number, or at least in form, in many species of plants and animals. Some of these are of a very general type and well understood, such as the sex chromosomes; others are of a very special type and little understood, like the sex-limited chromosomes of Sciara. In plants there is a vaguely defined group of chromosomes known by the term *supernumerary fragment*. Chromosomes of this kind, if indeed it is a kind, have been found in natural populations of some forty species of flowering plants (Langlet, 1927; Darlington, 1937, Table 16).

Observations of these fragment chromosomes have been for the most part too accidental for inference of any general properties in them. Their origin has been ascribed merely to breakage or deletion of an ordinary active member of the set. Yet enough common properties may already be inferred in them to encourage a further enquiry.

In *Tradescantia, Fritillaria, Lilium, Tulipa* and *Ranunculus* the fragments are small. They vary in number somatically and are capable of indefinite reduplication without any definable effect on the plant. They pair at meiosis by chiasmata with a frequency proportionate to their length and when multiple form multivalents. In the first three examples they show a relationship with the major chromosomes by pairing with them. In *Secale* the fragments are larger and they are, it seems, a constant type in the stocks of three continents. They do not pair with the major chromosomes. They must therefore be regarded as old-established accessories of the regular complement; so old-established indeed that they have lost their original relationship with it. All these fragments are what would generally be described as inert although, as we shall see, this provisional description conceals an analytical error.

In maize there are extra chromosomes of which we know something in all these respects. They are said to be of two recognizable types, B chromosomes somewhat smaller than the smallest standard or A chromosomes, and C chromosomes smaller still (Randolph, 1928 *a, b*). As many as twenty-five B chromosomes have been accumulated in one plant by selection without seeming to alter it in general or particular ways. These chromosomes are heterochromatic in the prophase of meiosis and sometimes at metaphase of mitosis (McClintock, 1933). They are present in plants of about a quarter of commercial varieties and genetic cultures. In each stock they have what appears to be an equilibrium distribution. Supernumeraries thus show, as in *Secale*, a contradiction between our failure to detect their genetic activity in individual plants and our ability to infer their genetic function from the population as a whole. The same situation has arisen with the extra X chromosomes of *Cimex lectularius*, which float in the population—here, however, with different equilibria in nature and in genetic cultures. The extra Y chromosomes described by Wilson in species of *Metapodius* seem to be in a like case (Darlington, 1939 *b*).

The B chromosome of maize therefore offers us the means of attacking several problems. Mechanically its indefinite reduplication allows of the exact comparison of crossing-over in bivalents and multivalents. Chemically its nucleic acid activity has to be compared with that of such chromosomes as the Y in *Drosophila*. Physiologically its indirect effects remain to be discovered. Evolutionarily the chances of its loss have to be equated against the advantages of its presence under different kinds of selection equilibrium. We will take the problems in this order.

2. THE B CHROMOSOME STOCK

We are indebted to Prof. G. W. Beadle for seeds of a genetic culture containing B chromosomes. Their inheritance is shown in outline in Table 1 to illustrate the origin of deficient types. Three plants appeared independently with one or two smaller B's which we shall designate b chromosomes. This high frequency of visible change is of general im-

Table 1. *Origin of b chromosomes from B's by deficiency, showing numbers in each plant*

Beadle's Stock
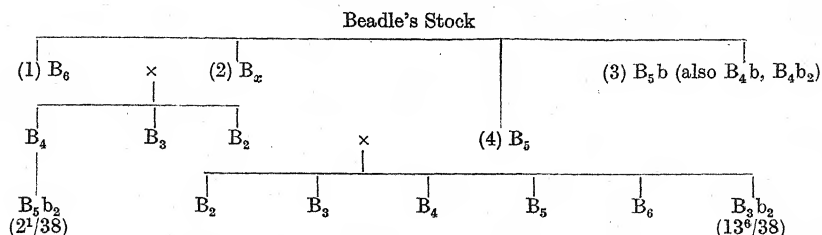
portance for the population genetics of B chromosomes. For the moment, however, it warns us that the classification of B and b chromosomes by their visible structure, which we necessarily use, is generic rather than specific. Our b chromosomes are presumably of three types and our B chromosomes may be equally diverse. We can, however, for our present purposes treat them as two groups.
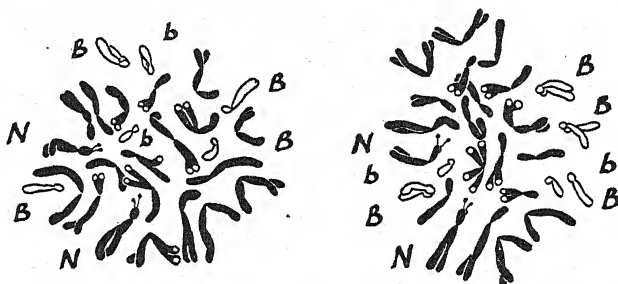
Text-fig. 1. Mitoses in root tips, 3/36: La Cour's 2 BE and gentian violet. $2n = 20 + 4B + 2b$. N, nucleolar chromosome, No. 6, with trabant. Note precocity of centromere in some B's. ×3000.

3. THE CONDUCT OF MITOSIS

The differential staining of B chromosomes has been described at various stages, at metaphase of mitosis by Darlington (1937, fig. 102), at pachytene by McClintock (1933), at diakinesis and first metaphase by Randolph (1928 a). Now La Cour (unpublished) finds that they show the differential Feulgen reaction during the mitotic resting stage which is

characteristic of heterochromatin. Dark-staining masses are seen in the nucleus equal in number to the B chromosomes seen at metaphase. Now, if B chromosomes are to be regarded as heterochromatic, it is of interest to recall that McClintock found a variable staining capacity of B chromosomes at pachytene just as with variable temperatures there is a variable nucleic acid content in the metaphase heterochromatin of
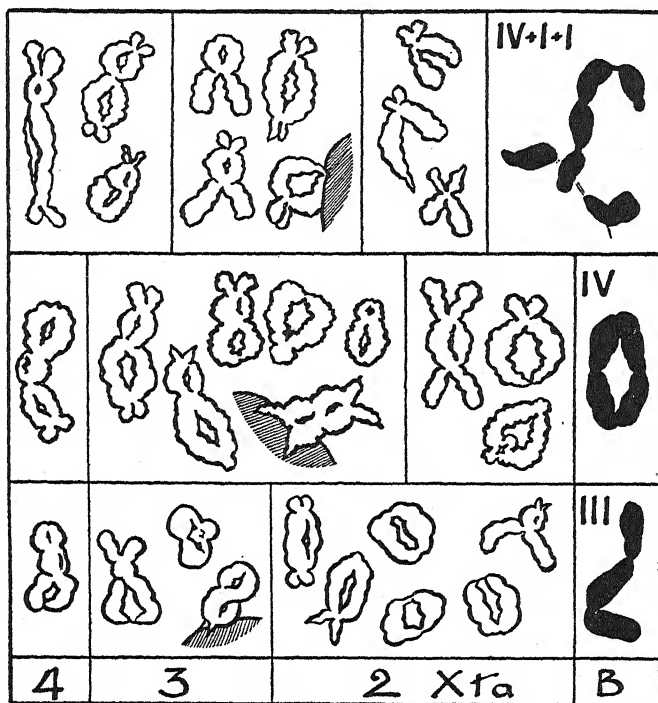
Text-fig. 2. Diakinesis in plants with 3, 4 and 6 B's, showing the chiasma distribution of A and B chromosomes and sticking of two unpaired B's. Acetic alcohol and iron acetocarmine preparation (as also following figures). × 2000.

Trillium (cf. Darlington & La Cour, 1940). These observations relate the B chromosomes of maize with an extra chromosome described by Fernandes (1939) in *Narcissus juncifolius*.

The difference in nucleic acid charge must not be confused with a difference in spiralization. As in *Trillium* this is the same for the two sorts of chromosome. At metaphase of mitosis both are contracted to about one fifteenth of their pachytene length.

Correlated with the excessive nucleic acid charge of B's is their stickiness and more even outline during prophase of meiosis (Text-fig. 2).

This property they share, as Longley (1937) points out, with the knobs scattered on the larger A chromosomes.

There is a second abnormality of mitosis which B chromosomes share in a lower degree with the extra fragments of *Tulipa galatica* (Darlington, 1937, fig. 17). That is the common habit of lying at the edge of the plate and of dividing at the centromere either before or after the major chromosomes (Text-fig. 1). These properties we are inclined to put down to the B chromosome having a weaker centromere than the rest, a centromere which, although usually sufficient for its smaller size, some-times fails to work in concert with the larger ones. The same evidence of weakness has been found in the *Secale* supernumerary (Hasegawa, 1934) and in *Uvularia* (Barber, 1940).

Hence we find occasional mitotic lapses in the B chromosome. From one of these we obtained material for a useful experiment. Plant 3/36 had some flowers with four and others with five B chromosomes in addition to one small b, as well as root-tips with two b's (Table 1).

Both B and b chromosomes in our plants are seen to have sub-terminal not terminal centromeres like those described by McClintock and Avdulov. We are inclined to suppose that this again means variation in the structure of the B chromosomes in the population, rather than misinterpretation on one side or the other.

The terminal centromeres, as well as the errors of mitosis shown by some B's in common with many other supernumeraries, they may well owe to their origin. Misdivision of the centromere is a common means of fragmentation and gives rise to terminal centromeres themselves fragmented and of weaker activity in various degrees (Darlington, 1940 a). On this view it is significant that the smallest fragments are liable to show the most frequent errors, while proportionately larger fragments like the B's seem to be relatively stable.

4. CHIASMA FORMATION IN B CHROMOSOMES

We already know from McClintock's work (1933) that B chromosomes pair at mid-prophase of meiosis but frequently fall apart at diplotene and are then unpaired at metaphase. Or, as we should say, after having paired at pachytene they do not always cross over and form chiasmata.

The chiasma formation of B's has to be considered in the light of the three respects in which they differ from the ten A's: shorter length, nearness of the centromere to one end, and heterochromatic properties.

Chiasmata are formed in three positions: S, short arm; P, proximally in long arm and usually very close to the centromere; and D, near the
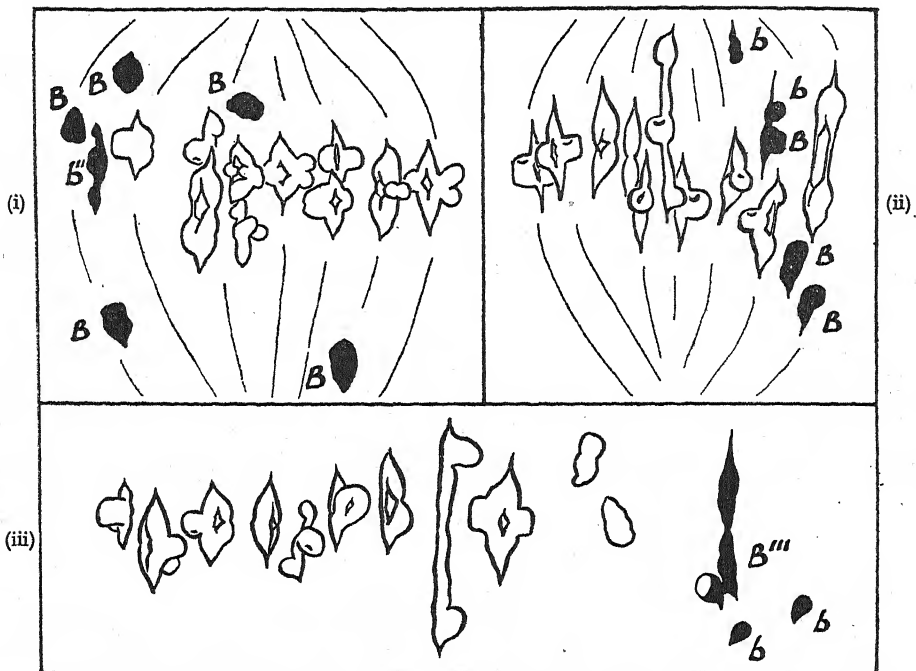
Text-fig. 3. First metaphases in plants with several B and b chromosomes, showing the position and orientation of univalents and multivalents. × 2000.
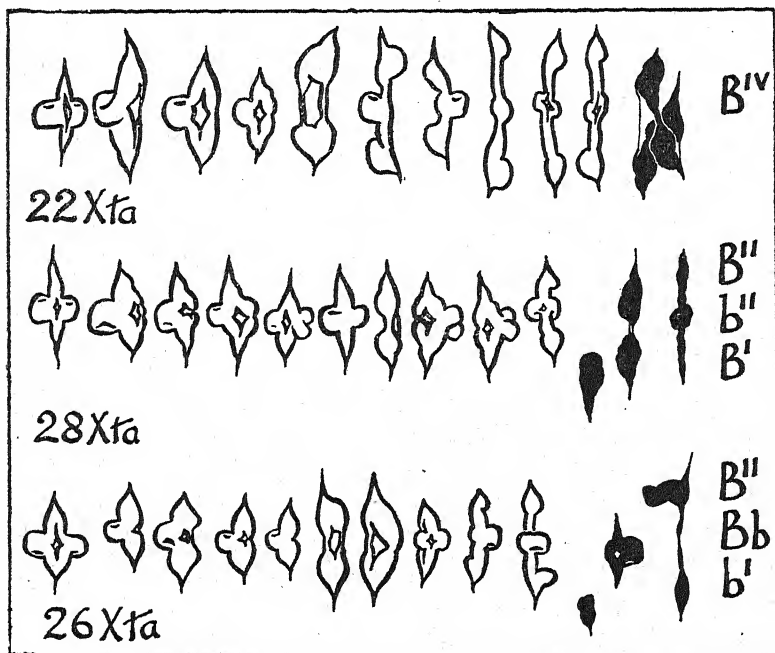
Text-fig. 4. First metaphases. Above with 22 A chiasmata, a ring quadrivalent of B's. Below with 28 and 26 chiasmata, two different arrangements of B's in 13⁶/38 with 3 B's and 2 b's. × 2000.

distal end of the long arm (Text-fig. 5 and Table 2). The formation of the S and P chiasmata distinguishes both B and b chromosomes from the A

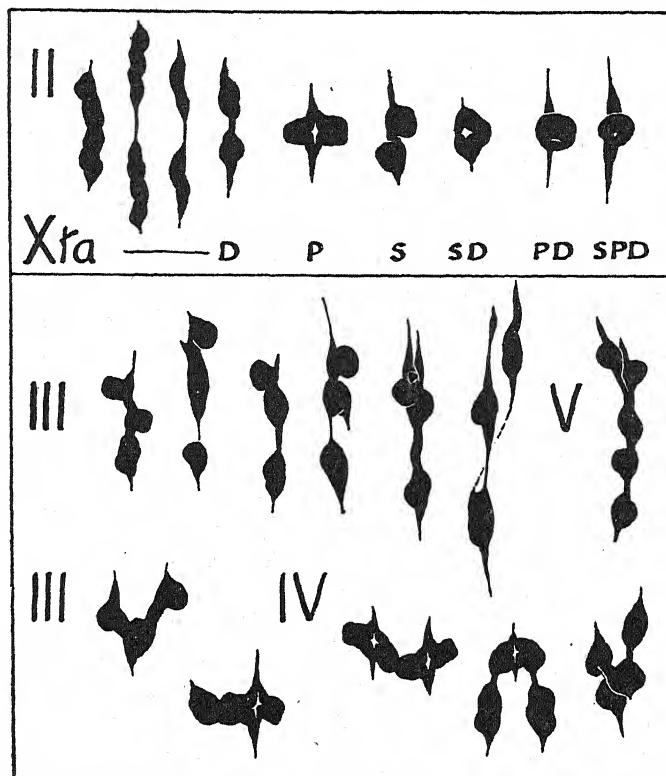Text-fig. 5. Bivalent and multivalent types of B showing the distribution of chiasmata in S, P and D segments and different co-orientations. × 2000.

Table 2. *Positions of chiasmata (Xta) in BB, bb and Bb bivalents*

Segments	S	P*	D	PD	SD	SPD	Bivalents	Xta	Xta/ bivalent
Symbols	100	010	001	011	101	111			
BB	1	46	13	0†	8	1	69	79	1·15
bb	0	0†	5	3	4	0	12	19	1·51
Bb‡	3	1	2	—	—	—	6	6	1·00

* Or SP.
† Illustrated, but not recorded in this sample.
‡ All illustrated and from 13⁶/38.

N.B. Sample of Bb and bb is selected, since these combinations do not occur without extra B's as well. In all groups bivalents alone are considered, without regard to the frequency of univalents.

chromosomes, for in these no chiasma is ever formed so close to the centromere as is possible and in fact most frequent in the B type. It

seems most likely that this change in position is due to the pairing being regularly proterminal, or beginning at the ends, in maize and to the centromere of the B being near to one end, so that it lies in the region of the highest crossing-over potential (Darlington, 1940 a). Whatever the mechanism, the B's show what is meant by the position determination of chiasmata (Mather, 1940).
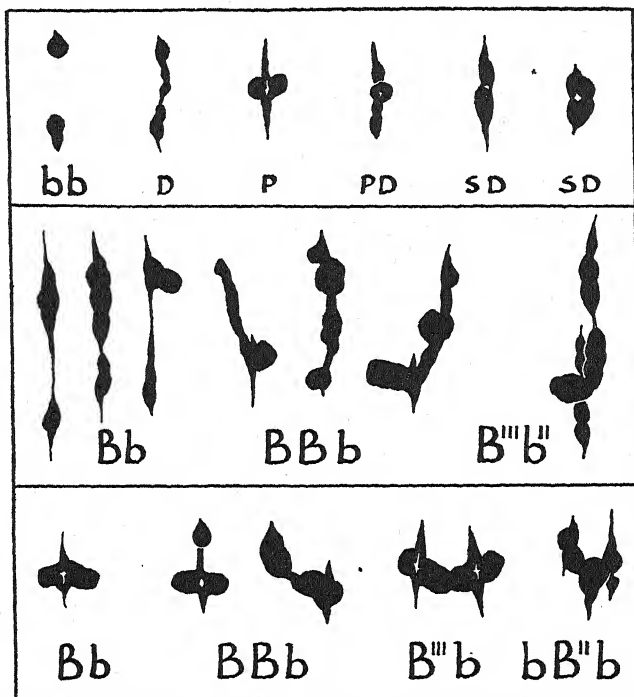
Text-fig. 6. Bivalent b chromosomes and pairing of b with B to give Bb bivalents with S, P and D chiasmata as well as multivalents. Cf. Text-fig. 7 for a diagrammatic representation. × 2000.

These chiasmata near to the centromere, together with the stickiness of the heterochromatin, lead to an unusual problem of interpretation. All bivalents with P-chiasmata appear also to have S-chiasmata, although simple S-chiasma bivalents are rare. Evidently the P-chiasma is so close to the centromere as to pull back the short arm on the other side of the centromere in the same way as happens in the separation of first division inversion bridges (Darlington, 1939 a). That there is not necessarily an S-chiasma is shown by multivalents of the BBb type of Text-fig. 6 (lower left-hand figure) where one B has an S-chiasma with a third chromosome, the b: the short arm without a chiasma is still con-

cealed behind the P chiasma. We have therefore made the minimum
assumption that, in the absence of contrary evidence, bivalents with the
P-chiasma have no S-chiasma.

B and b chromosomes pair with one another and form chiasmata
in the three positions comparable to those shown by simple BB and bb
bivalents (Text-figs. 3, 4 and 6). This means that the difference between
the two types of chromosome lies in the longer segment between the P
and D chiasmata: it consists in an intercalary deletion. Such a deletion

Origin of b from B chromosomes and their comparative chiasma formation
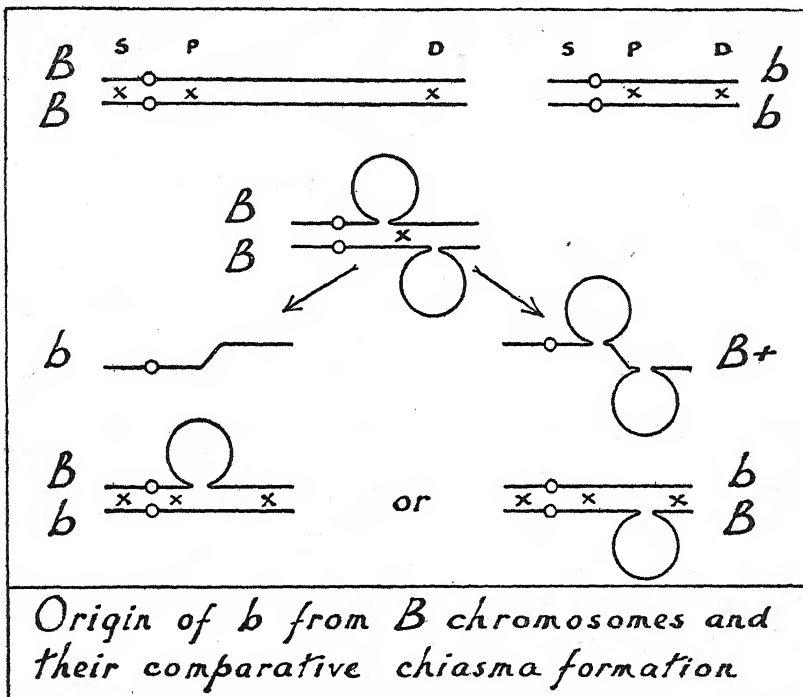
Text-fig. 7.

could take place through crossing-over between duplicated parts of two
identical B chromosomes. If it did so there would also be produced a
B + chromosome with yet another duplication such as we have not yet
identified (Text-fig. 7).

The chiasma frequency of B chromosomes in 2 B plants diverges
sharply from that of the A chromosome series (Darlington, 1934). B
chromosomes are two-thirds of the length of the shortest A chromosome
(no. 10) and should have a chiasma frequency greater than unity to be
in proportion. The frequency observed is 0·5.

A second divergence from the A type of pairing is shown by the variance which in all classes of B plants (with one exception, 3 B (ii)) is far higher: there is a more frequent failure of pairing, than would arise with a similar chiasma frequency in A chromosomes (Table 3). The obvious explanation of this excessive variance as well as of the lower aggregate frequency would be a partial failure of pachytene pairing. McClintock (1933) states, however, that B chromosomes pair fairly regularly. It follows that they pair differently from the A chromosomes, and indeed less effectively from the point of view of crossing-over potential. Such a difference has been described by McClintock as due to non-homologous pairing; it is favoured by the heterochromatic character of the B's. This kind of pairing we assume to take place by torsion instead

Table 3. *Distribution of chiasmata to AA and BB chromosomes, showing absence of positive or negative correlation within samples from single flowers of single plants*

	Chiasmata of BB	0	1	2	3	4
2 BB	Cells (11)	6	4	1	—	—
	Mean chiasmata of AA	23·16	23·75	28·0	—	—
3 BB (i)	Cells (9)	1	1	3	4	—
	Mean chiasmata of AA	21·0	21·0	22·3	22·8	—
3 BB (ii)	Cells (12)	1	7	5	—	—
	Mean chiasmata of AA	—	25·7	25·6	—	—
4 BB (i)	Cells (10)	1	4	5	—	—
	Mean chiasmata of AA	23	24·5	23·2	—	—
4 BB (ii)	Cells (15)	1	6	5	2	1
	Mean chiasmata of AA	24·0	25·8	26·0	25·5	22
5 BB, bb	Cells (20)	1	5	4	4	6
	Mean chiasmata of AA	23·0	26·0	26·0	27·0	25·3

of attraction. If the paired threads do not correspond they will never cross-over. And even the correctly paired parts, if very short, will not always cross-over. Occasional torsion pairing will therefore reduce the aggregate frequency of crossing-over and increase its variance.

The importance of torsion pairing in relation to crossing-over may be estimated in another indirect way. A chromosomes in our stocks show inversion crossing-over in 3 or 4% of cells (Text-fig. 8 A, B), and in one cell we have seen two bridges. A B chromosome bridge with fragment was found only once (Text-fig. 8 C). To judge from the frequency of deletions we have seen in B's, inversions should also be frequent. From the frequency of zip-pairing, which McClintock has found to override homology, inversion crossing-over should however be largely suppressed, for the correct pairing of an inversion loop is not always effected even in ordinary chromosomes. It is therefore worth recording that our single instance of inversion crossing-over was in a bivalent B in a plant with

only these two B's (Text-fig. 8 C). We found none in all the plants with multiple B's which McClintock has shown to have such frequent non-homologous torsion pairing at pachytene. We conclude that our single bridge results from homologous pairing of a genuine inversion and that the inverted non-homologous pairing does not lead to crossing-over.

A third divergence of B chromosomes from orthodox rules is shown by the absence of competition for chiasmata between A and B chromosomes such as Mather (1939) has found between A chromosomes alone (Table 3). Again we see that in the B chromosomes we are evidently
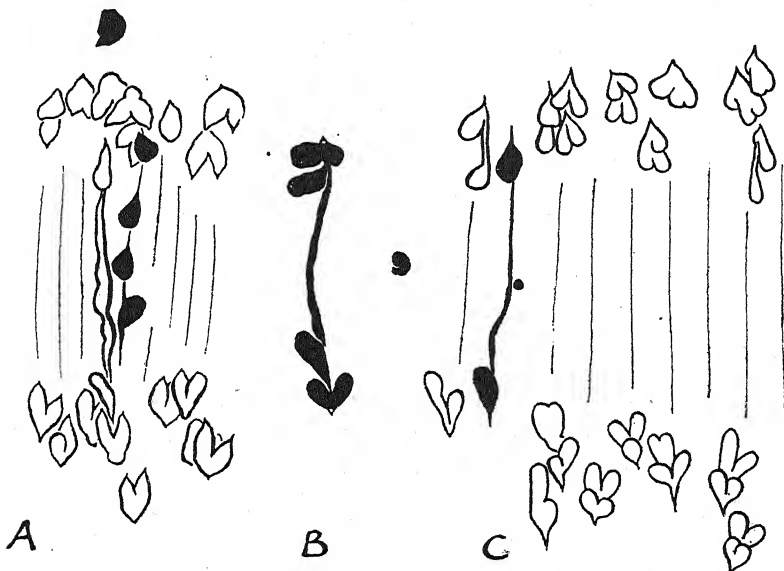
Text-fig. 8. First anaphases. A, A chromosome bridge and acentric fragment and lagging B quadrivalent. B, A chromosome bridge and acentric fragment. C, B bivalent bridge and fragment. ×2000.

dealing with a violently disparate system, one which owes its properties to its heterochromatic character.

The series of observations on 2 B, 3 B, 4 B, 5 B and 6 B cells, on the other hand, shows a concordance of a kind which no previous material has offered the opportunity of discovering, and a concordance, too, which does not seem to depend on the heterochromatin in their composition.

It will be seen that the high associations shown in Text-figs. 5 and 6 are scarcely compatible with the low chiasma frequency of B's in 2 B plants, and this is in fact true (Table 4 and Text-fig. 9). The higher

number of B's have a higher chiasma frequency. But this simple distinction is not enough. The even-numbered systems show a regular

Table 4. *Chiasma frequencies of B and b chromosomes present in different numbers (cf. Text-fig. 9)*

Plant	No. of cells	No. of B's	Chiasma frequency of BB		Chiasma frequency of AA per bivalent
			Per cell	½ Xta per chromosome	
2⁴/37	10	2	0·50	*0·50*	2·38
1/36	9	3	1·90	*1·27*	2·00
2⁶/37	12	3	1·42	*0·95*	2·57
2¹¹/37	10	4	1·40	*0·70*	2·37
2¹⁹/37	15	4	1·73	*0·87*	2·55
13⁶/38	20	5 (2b)	2·45	*0·98*	2·58
3/36	10	5 (1b)	2·60	*1·04*	2·42
	10	6 (1b)	2·60	*0·87*	2·45
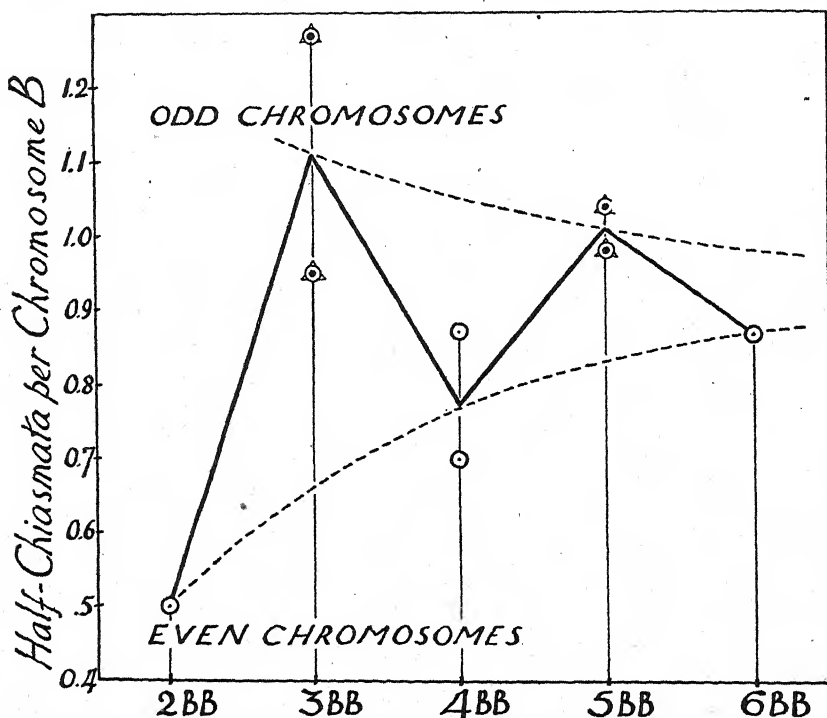
Text-fig. 9. Chiasma frequencies of B chromosomes in plants of different valencies (from Table 4).

increase, while the odd-numbered systems show an increase according to the proportion of odd chromosomes.

These two sources of increase—totality and oddness—can be understood in terms of their one common consequence, increase in the number of points of contact in pairing. In regard to oddness, these points are shown by changes of partner which are known to be more numerous in trisomics than in tetrasomics. In regard to totality, where the chromosomes are small, the points of contact are bound to be more numerous in tetrasomics than in the disomics, provided that the whole nucleus is not correspondingly multiplied (Darlington, 1940 b).

When active chromosomes are concerned a similar comparison can be made only with a balanced multiplication of the whole complement. The increase in the size of the nucleus then introduces a third factor, the reduction factor (Upcott, 1939), owing to the general slowing down of pairing. The inert B chromosomes therefore provide a test of a principle that can be tested in no other way and the result seems to depend on general mechanical principles and not on the special properties of heterochromatin.
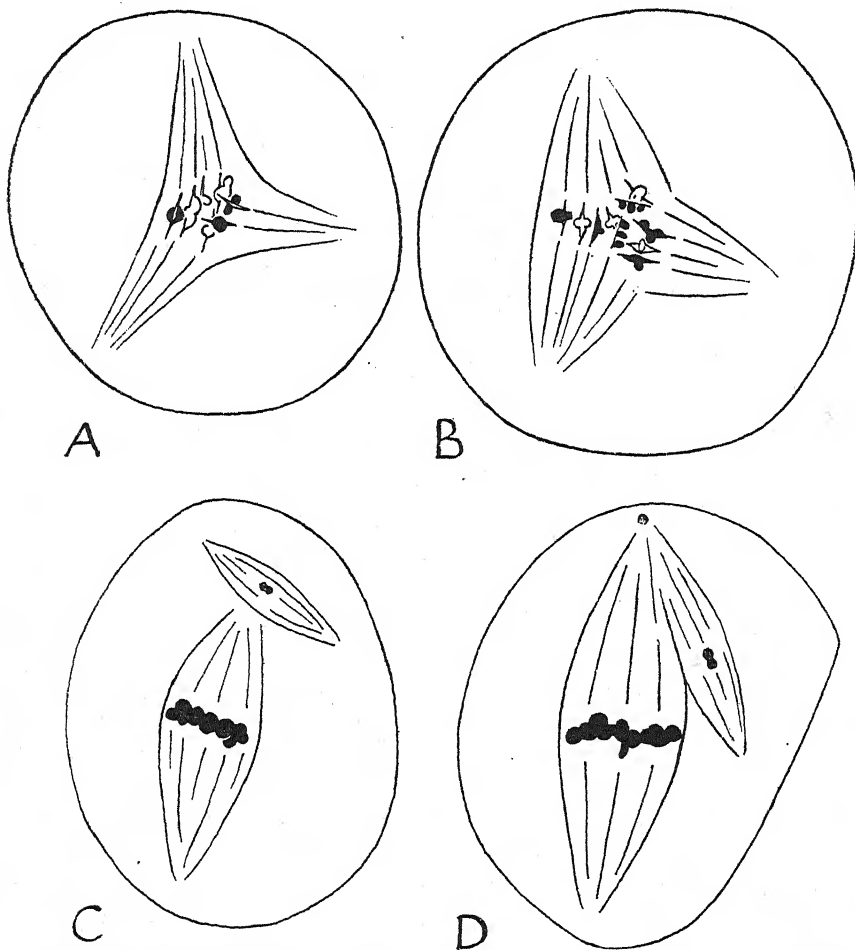
5. B CHROMOSOMES AND THE SPINDLE

Multivalent B chromosomes assume the linear, convergent and parallel forms of co-orientation proper to multiple figures at first metaphase (Text-figs. 4–6). The first of these is not found in A chromosomes for the simple reason that the large A's never have chiasmata near their centromeres so that their trivalents take up too much room on the spindle to allow of a linear co-orientation.

Univalent B's usually lie well removed from the equator, and they then take up a form not apparently found in A univalents or indeed in any unpaired chromosomes at meiosis (Darlington, 1934). The centromere is stretched in the axis of the spindle just as we saw in paired chromosomes with P-chiasmata and, in the same way too, the short arm is concealed so that the chromosome appears telocentric. Fig. 3 (i) illustrates a single exception to this rule.

The stretching of the spindle at anaphase pushes an unpaired B far beyond the paired chromosomes, so that at the second division it often forms a separate spindle. The structure of this spindle becomes clear when the material is preserved in 70% alcohol for some months. Successive stages then reveal its property of cohesion. The separate B spindle is too small to coalesce symmetrically with the main spindle, but the poles fuse (Text-fig. 10 C, D). Such arrangements no doubt reduce the frequency of loss of the B chromosome from the gamete nuclei.

This observation helps to explain the series of stages sometimes

found in the development of the first division spindle. Here a fusion of separate chromosome spindles probably takes place with some rapidity. Sometimes, however, it gives rise to a tripolar spindle which is more

Text-fig. 10. A and B, tripolar spindles at first metaphase. C and D, early independence and later cohesion of a B chromosome spindle at second metaphase. × 1000.

persistent (Text-fig. 10 A). And, rarely, the spindle seems to reach equilibrium in this position, so that an irregular anaphase will follow.

These examples of irregular cohesion show us the opposite side of the picture to that given by failure of cohesion as a particular genetic abnormality in grasses (Darlington & Thomas, 1937).

6. The selection equilibrium

The effects of failure of pairing and loss of B chromosomes at meiosis are first seen in the ensuing pollen grain mitosis (Text-fig. 11). There is in the first place a wide range in the number of B's, a range which may best be measured by the coefficient of variation (V/M). This value is 0·33 for the plant with four B's. It is evidently exaggerated by double reduction, or differential segregation at both meiotic divisions. This is shown by one haploid pollen grain which has more B chromosomes than
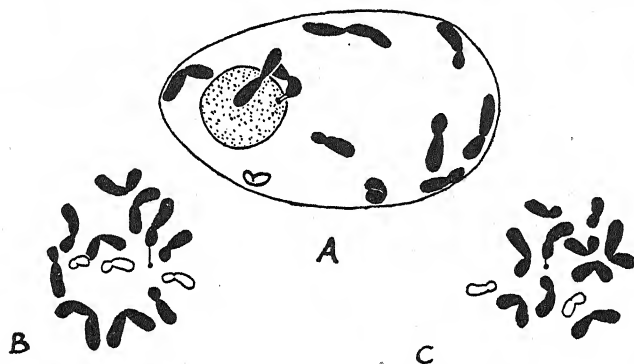
Text-fig. 11. Pollen grain mitoses with one, two or three B chromosomes from a 4 B plant. × 2000.

Table 5. *Loss and variance of B chromosomes between meiosis and first pollen grain mitosis in 4 B plant*

PG	10+0	+B	+2 B	+3 B	+4 B	+5 B	T	B's	M
No.	1	13	30	6	—	1	51	96	1·9

Total B's expected without loss: 102.
Chance of loss: 6%.
Variance (V): 0·63.
Coefficient of variation (V/M): 0·33.

its zygotic parent. Such double reduction occurs in fragments of *Tradescantia* (Darlington, 1929) and must be due to the passage of whole univalents without division into particular daughter nuclei of the tetrads, a not improbable event.

In the second place, the frequency of B's in the pollen grains has a lower mean than half the parental zygotic number and the reduction in mean may be taken as a measure of the chance of loss of each B, in this case a chance of loss at meiosis. This value is 6% in the plant with four B's (Table 5). It of course represents a fraction of the unpaired B chromosomes, a fraction greater than 6%.

These estimates are useful when we come to the next stage, the progeny of B plants. Reciprocal crosses show a higher loss on the female than on the male side (Table 6). Where the number of B chromosomes is odd rather than even, we expect, and we find, a higher coefficient of variation. We also find that the coefficient of variation is higher with B's coming from the male side than from the female side. And finally the coefficient of variation is lower with high-B than with low-B plants, a result attributable to the increase in chiasma formation, and the consequent greater regularity of pairing, that we have found with more B's.

One interesting combination of the results of O ×B and B ×O has been pointed out to us by Dr Mather: B ×B should have the sums of the means and variances of the first two crosses and the same V/M value. Agreement between the expected and observed distributions may be

Table 6. *Loss and variance of B chromosomes in transmission to progeny*

(0–2 B parents calculated from Longley (1927); 3–6 B parents from present results)

Parents...	0	B	2 B	3 B	4 B	5 B	6 B	Plants	B's	Loss %	M	V	V/M
B × O	109	58	3	—	—	—	—	170	64	*24·7*	0·38	0·27	**0·72**
O × B	76	22	15	—	—	—	—	113	52	*8·0*	0·46	0·52	**1·13**
B × B													
Obs.	105	91	55	14	—	—	—	265	243	*8·3*	0·92	0·81	**0·88**
Exp.	*114·3*	*93·9*	*43·3*	*12·9*	*0·6*	—	—	265	221·7	*16·3*	*0·84*	*0·79*	*0·94*
2 B × 2 B	6	19	13	30	5	—	—	73	155	*0*	2·12	1·47	**0·69**
? 3 B × 6 B	—	—	1	5	5	—	—	11	37	—	3·36	0·45	**0·13**
4 B × 5 B	—	—	2	2	2	2	1	9	34	*16·0*	3·78	1·95	**0·51**
5 B self	—	—	1	1	5	2	2	11	47	*14·0*	4·27	1·42	**0·33**

tested by the calculation of χ^2 if we group 3 B and 4 B classes: $\chi^2 = 4\cdot016$, D.F. $= 3$, $P = 0\cdot30$–$0\cdot20$, showing good agreement (Table 6).

So far we have taken the transmission of B's as resulting directly and without selection from their behaviour at meiosis, and our analysis does not allow us to contradict this simple assumption. Selection, as we shall see, might very well have modified the results, but there is no evidence of it in the experiments.

Now, however, with the knowledge of what happens within families of uniform parentage, we can turn to the populations. Randolph's determinations (1928 a) were made on two classes, genetic cultures and commercial varieties (Table 7). Four of the cultures are F_2 and F_3 families and these show the lowest coefficients of variation. The fifth, perhaps less inbred, shows the highest value. The varieties, which must be less inbred than the cultures but perhaps more selected, show intermediate values. But what is remarkable is that the values of both cultures and varieties are so little higher than in the single families with corresponding means.

They have for the most part the same unimodal distribution with a scarcely increased V/M value. Such a situation can be maintained only by selection in favour of an optimum number of B chromosomes in each stock.

There is a second and entirely independent source of evidence of selection pressure acting on the occurrence of B chromosomes. At every meiosis, as we have seen, there is a certain chance of loss varying as between male and female and as between high and low, and odd and even B plants. Furthermore, the B's suffer a continual chance of loss of parts as we have seen in the origin of the deficient b's. Nevertheless, the B chromosomes continue in the population and have continued for a long period. This is shown (as in *Secale*) equally by their present lack

Table 7. *Coefficients of variation in the number of B chromosomes in cultivated populations (after Randolph, 1928a)*

	No. of B's ...	1	2	3	4	5	6	7	8	9	n	M	V	V/M
	Culture													
21	*sŭ Tu tu*	4	5	—	1	—	—	—	—	—	10	0·80	0·84	1·05
15	(F₂) *B, ts₂ × Ts₂ts₂*	1	4	5	1	—	—	—	—	—	11	1·55	0·67	0·43
16	(F₂) *B g Tu tu*	2	5	6	7	—	—	—	—	—	20	1·90	1·04	0·55
11	(F₃) *a C R Pr Su lg*	1	1	5	2	4	1	—	—	—	14	2·71	1·92	0·71
1	(F₃) *A C R Pr Su*	—	—	3	2	4	1	—	—	—	10	3·30	1·12	0·35
	Variety													
	H. G. Nugget	6	2	—	—	—	—	—	—	—	8	0·25	0·21	0·84
	G. Bantam	10	6	3	—	—	—	—	—	—	19	0·63	0·58	0·92
	N. Y. S. Flint	2	2	4	2	—	—	—	—	—	10	1·60	1·04	0·65
	Bl. Mexican	2	6	8	12	5	4	4	1	1	43	3·2	3·30	1·03

of apparent relationship with the normal chromosomes and by their wide distribution in unrelated stocks. There must therefore be a selection pressure acting on whole plants to increase the number of B's in the population exactly equal to the selection pressure acting on the chromosomes in the cells to reduce their number. We thus have two selective forces acting at different levels of integration and producing in different stocks different equilibria. Indeed the measurement of the known mechanical loss is a measurement of the unknown populational gain.

There is thus not merely an optimum number of B's in each population but that optimum number is greater than the mean number observed.

A measurable selection in favour of B's means that these supposedly inactive chromosomes have an activity and a measurable activity. That activity would seem, from their heterochromatic properties, to depend on their special nucleic acid metabolism. This view is supported by Longley's observations (1938) that in thirty-three primitive maize stocks

in the U.S.A., thirteen have B chromosomes and these are more frequent where the A chromosomes have fewer heterochromatic knobs. And this combination in turn is more frequent in regions more remote from Mexico. B chromosomes occur in *Euchlaena mexicana* but their presence is to be attributed to contamination with maize and not to their independent origin in this species (Longley, 1937). Thus it seems that, as the cultivation of maize has progressed, the B chromosomes have taken over and enlarged the function of the knobs; and in doing this they have provided a more elastic means of regulating the nucleic acid metabolism of the nucleus as a whole.

Whatever the activity of the B chromosomes may be, it is evidently of a different kind from that of the A chromosomes, and should therefore be subject to different laws of evolutionary change. That this is so we see in two ways. The appearance of the deleted b chromosome three times in our experiments shows that original and spontaneous changes are more frequent than in the A chromosomes. And, further, since the B chromosomes as wholes are not indispensable, the loss of their parts cannot be immediately and seriously deleterious. Nevertheless, we see that the B chromosomes are preserved at about the same size in all cultures and at all times, since they were first found by Kuwada in 1911, and the gain in size which we might expect as the reciprocal of loss does not seem to occur. There are therefore conditions limiting the effective variability of the B chromosomes, conditions which are, mechanically, more stringent than those that apply to the larger active chromosomes. We would suggest that three such conditions can already be specified:

(i) That reduction in size leads to too frequent loss at meiosis for the small b's to survive as alternatives to large B's, although the B's may themselves have begun as even smaller bodies.

(ii) That increase in size leads to too frequent loss at mitosis for similar survival. The large B already has a sub-efficient centromere, as shown by its irregularity at mitosis. Its centromere will not carry a greater load.

(iii) That internal rearrangements not leading to change in size are largely indifferent, since the parts of the B are scarcely differentiated in function. We must therefore expect considerable rearrangements, such as are indicated by McClintock's determination of a terminal centromere in her stocks and our determination of an intercalary one in ours, without any great change of size. Their kind and frequency will repay study in crosses of different B stocks such as seem in the past to have been somewhat miraculously avoided.

It is worth while knowing something of these principles, for the B chromosomes of maize are just one example of an evolutionary progression of which we are beginning to see the steps. In many species we see new fragment chromosomes appearing from time to time. Most of these probably come to nothing. In *Fritillaria, Tradescantia* and *Ranunculus* we see them reduplicated to such an extent that they must be taken to have acquired a use in the species, the race or the clone. In *Secale* they are so widespread as to have maintained this use for a long time. In *Zea Mays* they have established special equilibria in special varieties. These different equilibria are a symptom of the rapid adjustment of this rapidly evolving species, or genus as we may say, since *Zea* as a genus owes its existence to human selection of *Euchlaena*.[1] Whether in fact the urgent selection practised by man in favour of bigger and better corn, demanding as it has done the reconstruction of cell activity on a new level, is responsible for the spread of B chromosomes in certain stocks, even when transplanted back to *Euchlaena*, we cannot yet say. But it is a question to which maize breeders can supply the answer.

Turning to animals, we see in the Heteroptera an even more advanced condition of the subinert supernumerary. In some species, like *Nepa cinerea*, it has acquired constancy of reproduction, while in others, like *Cimex* and *Metapodius*, it varies in number. It is indispensable for the population although not yet for the individual. In this group parts of the sex chromosomes have been used as a basis for the supernumerary system, and it fits them well mechanically on account of their special trick of segregation. But the physiological implications are the same as in plants, where ordinary chromosomes are used as the basis of supernumeraries: they provide a new supply of heterochromatin which is readily adjustable to a changing economy.

Our conclusion is therefore clear. Some species are conveniently organized with their hereditary materials in one type of chromosome. Others arrive at a division of labour. The physiology of this division and its mechanical and evolutionary stability are problems that can be handled by the comparison of other species that are undergoing a reconstruction of their cell life.

7. SUMMARY

1. B chromosomes of *Zea Mays* are short heterochromatic chromosomes hitherto supposed to be inert but existing in equilibrium in many genetic cultures and varieties.

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[1] A view we must continue to hold, in spite of Mangelsdorf & Reeves (1939).

2. Their frequency of crossing-over is less than proportionate to their length by the A chromosome standard, and its distribution is different. B chromosomes regularly form chiasmata closer to the centromere than is possible in A chromosomes. This is due to the position of the centromere which is nearer one end than in the A type.

3. Their unlimited reduplication makes it possible to show that their crossing-over frequency is increased by odd numbers. This effect is due to more frequent changes of partner at pachytene, on the anchorage principle.

4. B's are liable to irregularity at mitosis (owing to deficient centromeres) and loss at meiosis (owing to non-pairing). They are also specially liable to deletion (owing to heterochromatin content) and then give smaller b chromosomes.

5. A double selection pressure thus acts to reduce their size and frequency in the population and must be compensated by an equal and opposite selection in their favour.

6. The equilibrium that is reached implies that mechanical loss in the population of cells is equal to the physiological gain in the population of organisms.

7. Thus the cell observations measure the total opposite selection pressure and reveal a population, such as we might expect in *Zea Mays*, under high *selectional stress*.

8. Longley's observations may be·taken to indicate that under artificial selection B chromosomes are replacing the heterochromatic regions of A chromosomes in the nucleic acid metabolism of the cell.

9. Similar subinert supernumeraries in other organisms fall into an evolutionary progression, indicating division of labour in a hitherto disregarded function of the chromosomes: the maintenance of their own mitotic economy.

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EXPLANATION OF PLATE 9

Figs. 1–4. First metaphases in pollen mother cells of *Zea Mays* with B chromosomes. × 2000.

Figs. 5 and 6. Second metaphases. × 1000.

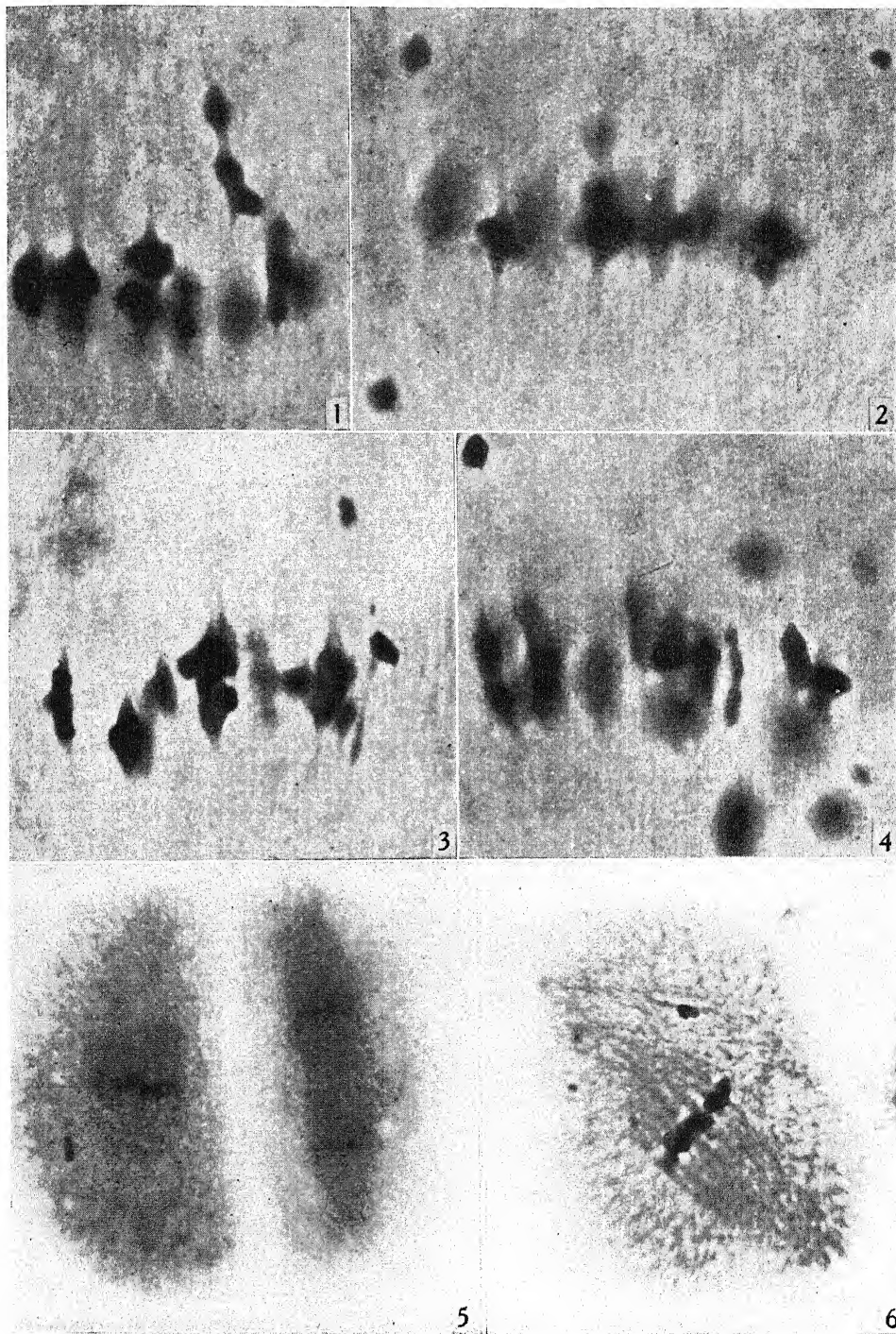Fig. 1. Trivalent B (third in Text-fig. 5).

Fig. 2. Univalent B and b chromosomes.

Fig. 3. Bb bivalent with S chiasma (third in Text-fig. 6) and univalent b chromosome.

Fig. 4. bb bivalent with D chiasma (Text-fig. 6) and B univalents off the plate.

Fig. 5. B chromosome lost in the cytoplasm.

Fig. 6. B chromosome with spindle cohering to the main spindle (Text-fig. 10 D).

SPONTANEOUS CHROMOSOME CHANGE

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(With Plate 10 and Twenty-three Text-figures)

CONTENTS

1. TIME AND PLACE

THE permanent changes in chromosomes which are responsible for variation and evolution are said to be "spontaneous" or "natural". They seem to occur independently of any specific action external to the plant or animal. And we are only beginning to understand the internal actions which may be responsible for them. What we know comes from the cytology of gross structural changes rather than from the genetics of fine heritable changes or gene mutations. For with the gross changes we can follow the steps of the process at the next cell division, while with the gene changes these steps have to be inferred less directly.

The event of natural breakdown is rare, but its consequences are common. We have therefore been chiefly concerned with classifying these consequences. They are seen naturally in the form of hybridity, and we are able to compare the kinds of natural hybridity with those arising from artificially induced changes. We find they agree. Determination and mechanism are less accessible. For artificial change, Muller and Delbrück have recently clarified the position. For spontaneous change, on the other hand, we are still very much in the dark. Recent work, however,

has shown that within the limits of natural conditions unimagined possibilities occur for structural change in the chromosomes. Such possibilities arise under three general types of condition: first, extreme natural conditions, e.g. temperature; secondly, special genotypic conditions, e.g. mutation, hybridity or segregation; thirdly, differentiation

Table 1. *Primary and spontaneous chromosome breakage or union at mitosis*

(1) All tissues	*Zea Mays* "**st**"	$2x$	Sticking at anaphase	Beadle, 1932
(2) Root tip				
(i) normal	*Paeonia peregrina*	$4x$	18 **b** in 17 cells also 1 C_0 and 1 C_1t	Barber, unpub.
	Paeonia corallina	$4x$	20 root tips: 1 **b** + **f**	,, ,,
	Pisum sativum	$2x$	**b** + **f**	Rutland, unpub.
	Allium Cepa	$2x$	Sticking at anaphase	Callan, unpub.
(ii) chilled	*Trillium grandiflorum* *T. stylosum* $2x$		127 cells: 31 **b** (heterochromatin)	Darlington & La Cour, 1940
(3) Before meiosis	*Chorthippus* hybrid $2x\male$		Sticking at anaphase	Klingstedt, 1939
	Hosta undulata	$2x$	Various breaks and joins	Akemine, 1940
(4) Pollen grain	*Tradescantia*	$3x$	Sister chromatid bridges	Upcott, 1937*a*
	Hyacinthus	$2x$		
	Tradescantia	$2x, 3x$	**b** + **f**, $C_2 + C_0$	Giles, 1940
	Paris	$4x$	Knot formation	Husted, 1937
	Pancratium	$2x$	,,	,,
(5) Pollen tube				
(i) fresh	*Tulipa cretica*	$2x$	1–3 bridges	Upcott, 1936
(ii) aged	*Kniphofia rufa*	$2x$	,,	Barber, 1938
	Paeonia Veitchii	$2x$	,,	,,
(6) Tapetum (polyploid)	*Podophyllum umbellatum* $2x$		Connecting strands at telophase	Cooper, 1933
	Lilium canadense	$2x$	,,	,,
	Paeonia Whitmanniana $4x$		1–10 bridges, no **ff**	Barber, unpub.
(7) Endosperm	*Zea Mays*, mosaic types		1–3 **b** + **f**	Clark & Copeland, 1940

Note: abbreviations are explained in the appendix.

within the organism leading to abnormal conditions in *blind* or disintegrating tissues.

One group of these changes may be separated from the rest by their depending on the special activity or inactivity of the centromere, namely misdivision (Darlington, 1940) and the formation of diplo-chromosomes (White, 1935). The conditions of these changes can be approximately defined. The undefined group are shown in Table I.

These observations concern the gene string and fall into three classes: (i) bridge formation due to terminal union of sister chromatids;

(ii) intercalary sticking of chromatids at anaphase, sometimes leading to fragmentation; (iii) breakage resembling the artificial or X-ray type.

In this last group we must define what we mean by the X-ray type in order to discover whether the artificial breakage can indeed serve as a copy of a natural event. We mean first that the chromosomes are broken during the resting stage and some of their broken ends later reunite in new ways, according to Stadler's *breakage-first* principle. Secondly, we mean that the chromosomes may be broken before or after they divide, to give chromosome or chromatid breaks, on Mather's *time-of-split* principle (cf. Muller, 1940).

These two principles with their separate and joint implications must be the basis of interpretation equally of induced changes and of any spontaneous ones which may seem to obey the same rules. What are these implications? The following seem to be the least we can assume:

(i) When a chromosome is broken, the two parts may rejoin as they were before. This is the principle of *restitution* now generally accepted.

(ii) All pairs of broken ends have an equal and non-specific mutual affinity, so that the type of reunion, whether old or new, will depend on positions alone. This assumption may prove to be unsound, but it must be taken as a starting point in analysis.

(iii) The proportions of the original breaks which will be observable (*a*) by persisting to the next mitosis and (*b*) by rejoining to give new combinations will depend in opposite ways on the time of breakage and the condition of the nucleus. The relationship of quantity of radiation with observed breakage, unlike that with observed gene mutation, cannot be a direct one either simple or exponential.

(iv) Chromatid reunion, new or old, will be capable of following chromosome breakage, and since new reunion will be slower than restitution, observations of new unions from timed breakage will antedate the time of split.

The variety of these implications requires that we shall use every possible means to separate the processes at work. One of these means, perhaps the chief of them, is statistical. The distribution of observable breaks and rejoins, like that of original breaks, must have a characteristic variance from cell to cell which will perhaps alone discriminate between ionization breaks and natural breaks.

The special value of a study of spontaneous change is that while the method of breakages is different, the method of reunion may well be the same. Indeed where there is a detailed agreement between the spontaneous and X-ray changes we have the right to assume that we are

dealing with a property of reunion common to all broken chromosomes rather than of breakage independent of its agency.

The material of the present study belongs to classes 2, 3 and 5 in Table 1. A further genetic subdivision is useful in indicating the conditions of structural instability:

I. Old clone, sterile: *Tulipa orphanidea*   2x

II. Numerical hybrids:

    Tradescantia "*virginiana*"   3x
    Tulipa saxatilis   3x
    T. praecox   3x
    Tulipa, "Inglescombe Yellow"   3x
    Hyacinthus, "Yellow Hammer"   3x

III. Mutants or segregates (conditioned partly by environment):

    Hyacinthus, "Wm. Mansfield"   2x
    Tulipa cretica   2x
    T. Clusiana   2x
    T. fragrans   2x
    T. sylvestris   4x

External as well as genetic conditions proved to be important in the last group. Yet the conditions have lain, so far as we can tell, within natural limits in all observations. Even for pollen tubes, which are artificially cultivated, they are not such as are usually attended by abnormality. The case of *Tulipa fragrans* (a form of *T. australis* brought from Algeria by the late W. C. F. Newton) will be specially described.

Two triploid forms of *Tradescantia* were examined. One, *T. brevicaulis*, showed no changes; the other, *T.* "*virginiana*" from the Lyons Botanical Garden, showed extensive changes every year for three years. The percentage distribution of chromosome numbers in their mitoses is shown in Table 2. They show the very slight loss of odd chromosomes at

Table 2

	Total	6	7	8	9	10	11	12	Mean	Loss
Tulipa brevicaulis	150	0·6	10·0	20·0	40·7	24·0	4·7	0·0	8·88	4·0%
T. "*virginiana*"	200	1·0	9·5	24·0	28·0	30·0	7·0	0·5	8·99	0·3%
Binomial		1·5	9·4	23·5	31·3	23·5	9·4	1·5	9·00	—

meiosis which is characteristic of the tightly packed pollen mother-cells in *Tradescantia* (Upcott & Philp, 1939). In all triploids, as Upcott (1939) has pointed out, the degree of spiralization of mitotic chromosomes is variable in the pollen. This has already been illustrated in the triploid *Tradescantia* clone (Darlington, 1937*a*).

2. Pollen in General

In triploids, secondary structural abnormalities of three kinds can arise in the pollen grains as a consequence of primary changes at meiosis (Giles, 1940). The value of triploids is that the fatal deficiency which is bound to follow various losses in a diploid can be covered by extra chromosomes in the pollen grains of a triploid. The first of these derived changes, the dicentric chromosomes from non-disjunction in trivalents following inversion crossing-over, have already been illustrated in *Tulipa praecox* (Upcott, 1937*b*, fig. 52). We now see them in *T. saxatilis* (Text-figs. 1*b*, *c*, 4*b*) and Inglescombe Yellow (Text-fig. 4*a*).
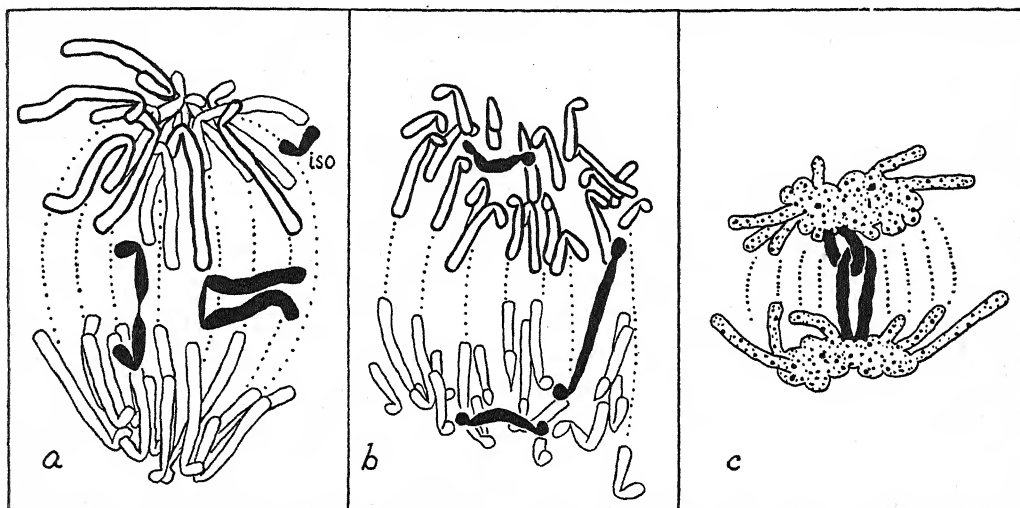

Text-fig. 1. Pollen grain anaphases in triploid tulips. *a. Tulipa praecox*: two **SR** (sister reunions) and one iso-chromosome. Feulgen smear. ×1500. *b* and *c. T. saxatilis*: separation of dicentrics (**C₂**) without and with interlocking. One **SR** chromosome in *b*. Acetocarmine. ×1000.

A second derived change is the formation of iso-chromosomes from misdivision at meiosis (Darlington, 1940, and Text-fig. 1*a*). The third change is the production of loop chromatids by sister reunion. The loops give single bridges at anaphase (Text-figs. 1*a*, *b*, 2). They arise from breakage of bridges at meiosis, which is also indicated by occasional deficient metaphase chromosomes (Text-fig. 5*a*).

In diploids the problem is different, for with them a broken arm will usually be deficient or duplicated and therefore deleterious. This difficulty is avoided where meiosis has been suppressed, and the changes are then preserved in the pollen grain (Text-fig. 10*b*). The hyacinth "William Mansfield" is not only diploid; it has no inversion bridges at meiosis.
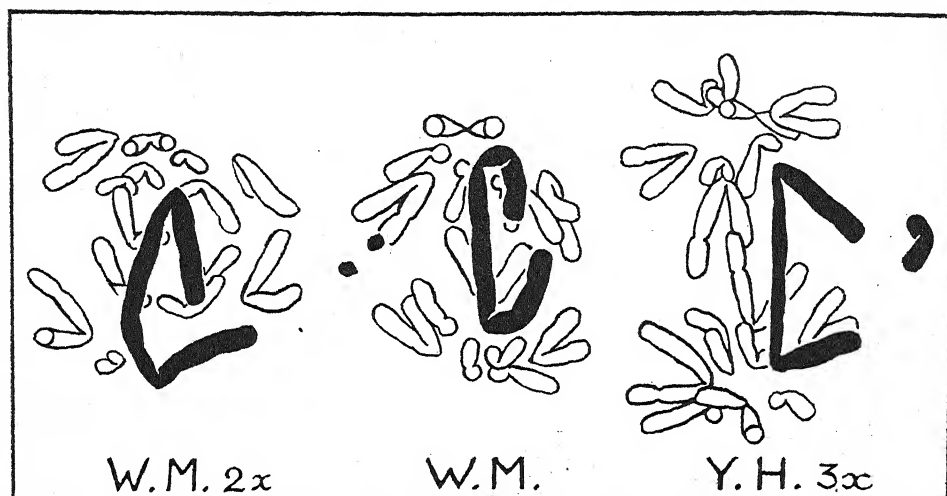
Text-fig. 2. Anaphases in diploid and triploid *Hyacinthus* showing **SR** with and without
prior breakage. × 2600.
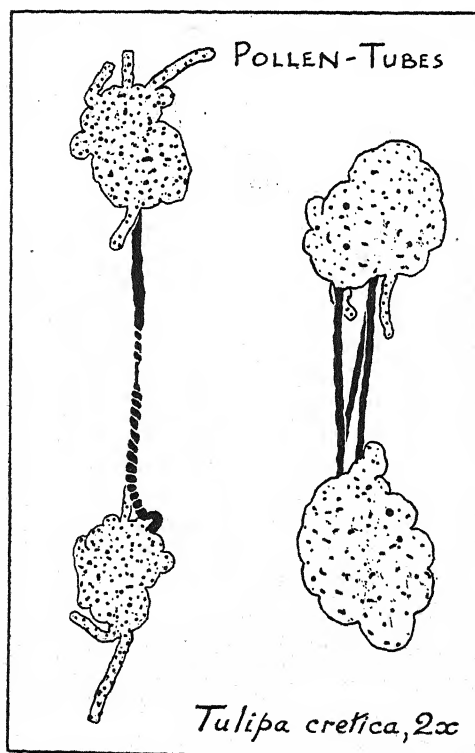
Text-fig. 3. Bridges from **SR** in pollen tubes. × 500.

Yet sister chromatid fusion occurs, both with and without fragmentation (Text-fig. 2). Apparently we have not only fusion following spontaneous breakage but also in some cells spontaneous fusions of established ends. Whether some of the bridges in triploids also are due to such spontaneous fusion must therefore be left open.

Barber (1938) has already shown that with ageing, established ends of sister chromatids will fuse in the pollen tube. We now find that such fusions can occur in fresh pollen, in a diploid species, and give not only even numbers of bridges such as might arise from the breakage of meiotic dicentrics but also odd numbers (Text-fig. 3). Since sister fusion of heterochromatic ends can be induced by nucleic acid starvation in cold, it is not surprising to find it also occurring spontaneously (Darlington & La Cour, 1940).

If sister reunions of chromatids are sometimes due to primary changes, their non-sister reunions must always be so. They must have arisen from breaks after meiosis and usually after the split of the chromosomes in the following resting stage. The clearest of these are interchanges of chromatids. They give the metaphase pseudo-chiasmata which are so significant in showing that it is the condition of the centromeres, not the exchange of chromatids, which determines co-orientation at meiosis. An "asymmetrical" interchange (Text-fig. 5 b) will give single dicentric and acentric chromatids at anaphase. The two centromeres of the dicentric chromatid may pass to the same pole (Text-figs. 4 c, 5 b, c) or to opposite poles (Text-figs. 5 c, d). In the latter case the inequality of the free arms is the only proof at anaphase that we are not dealing with a sister reunion such as will give merely the kind of loop chromatid assumed in Text-fig. 2.

Again, when a high amount of breakage occurs, there can be no doubt of its primary character. In the triploid *Tradescantia* this is all the clearer since most of the reunions are chromatid reunions (Text-fig. 6). In one cell (6 a) three branched chromosomes arose owing to the independent union of sister ends with different chromatids. In another cell (6 c) there are four examples of sister reunion, three in monocentrics, one in the long acentric. Such structures are due to chromatid reunion following chromosome breakage. This sort of coincidence is peculiar, as we shall see, to the circumstances of spontaneous or internally determined changes.

One particular kind of abnormality of this *Tradescantia* takes us right over the border-line from coincidence to specificity. Like misdivision in *Fritillaria karadaghensis*, it is an abnormality of the centromere. At an ordinary anaphase the chromatids are subjected to a strain between the centromere and the point of separation, a mild form of the Klingstedt
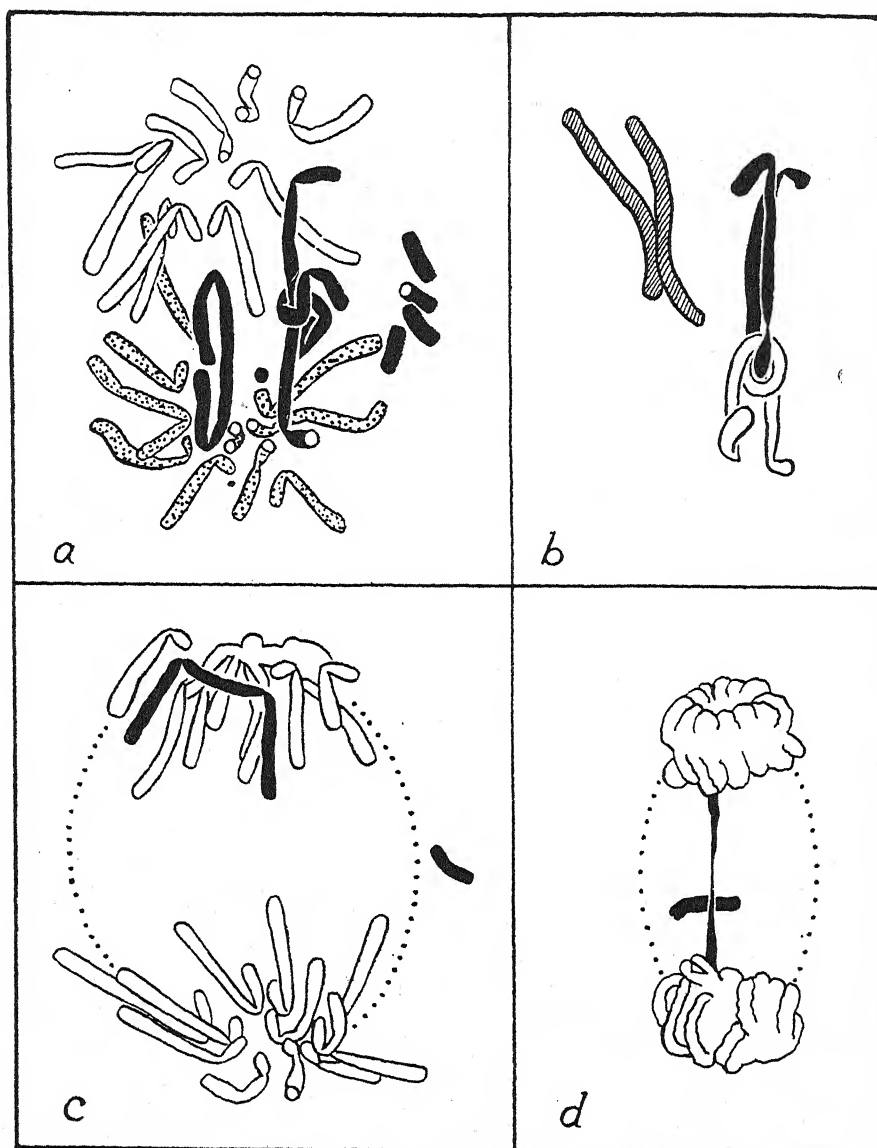
Text-fig. 4. Anaphases in pollen grains of triploid tulips. *a*. Inglescombe Yellow: dicentric and two acentric chromosomes. One broken chromosome with **SR** of both parts. 2 BE + gentian violet. × 2600. *b*. *T. saxatilis*, dicentric and unrelated acentric. Acetocarmine. × 2000. *c*. As *a*, with chromatid interchange. *d*. *T. praecox*, result of *a* or *c* with cross-segregation of two centromeres to give a bridge. Acetic alcohol with Feulgen. × 700.

effect (Text-fig. 7). In *Tulipa silvestris* we have found the direct consequences of this in one pollen grain. Every chromatid has broken near, but not next to, the centromere during anaphase. The effect (which is
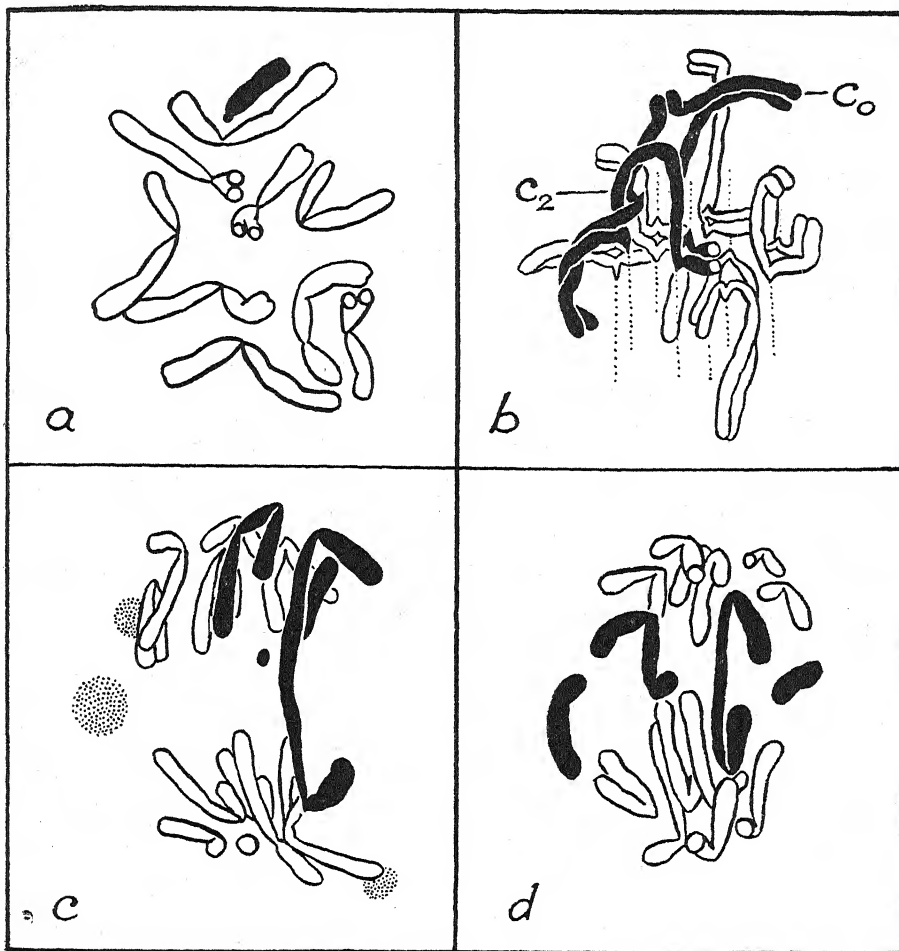
Text-fig. 5. *Tradescantia virginiana*, 3x. 2 BE + gentian violet. *a.* Deficient chromosome. *b.* Asymmetrical interchange of chromatids giving dicentric and acentric chromatids at metaphase. *c* and *d.* Consequences of the situation shown in *b* at anaphase; eight centromeres in each nucleus. × 2000.

illustrated by three photographs in the plate) resembles diminution in *Ascaris.*

Independently of any anaphase strain, the centromere breaks down in some pollen grains of our *Tradescantia.* The breakdown may even take
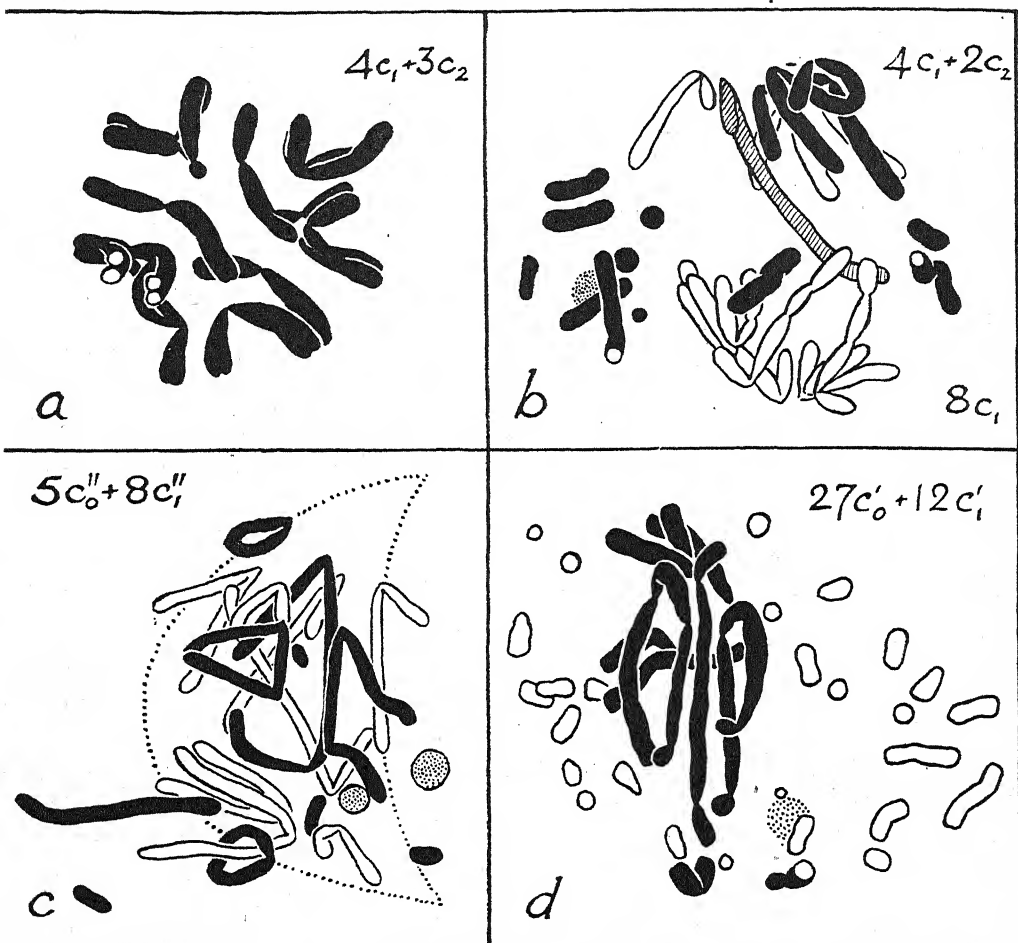
Text-fig. 6. Extreme examples of *T. virginiana* (3x). *a*. Three branched chromosomes. *b*. Ten acentric chromatids, two dicentric chromatids. *c*. Five acentric chromosomes and 8 monocentrics. Note: interlocked monocentric ring has escaped on side. *d*. Complex changes. ×2000.

Text-fig. 7. Pollen grain anaphase in triploid *Tradescantia* showing the tension to which chromatids are sometimes subjected by delay in lapse of their attraction. ×2400.
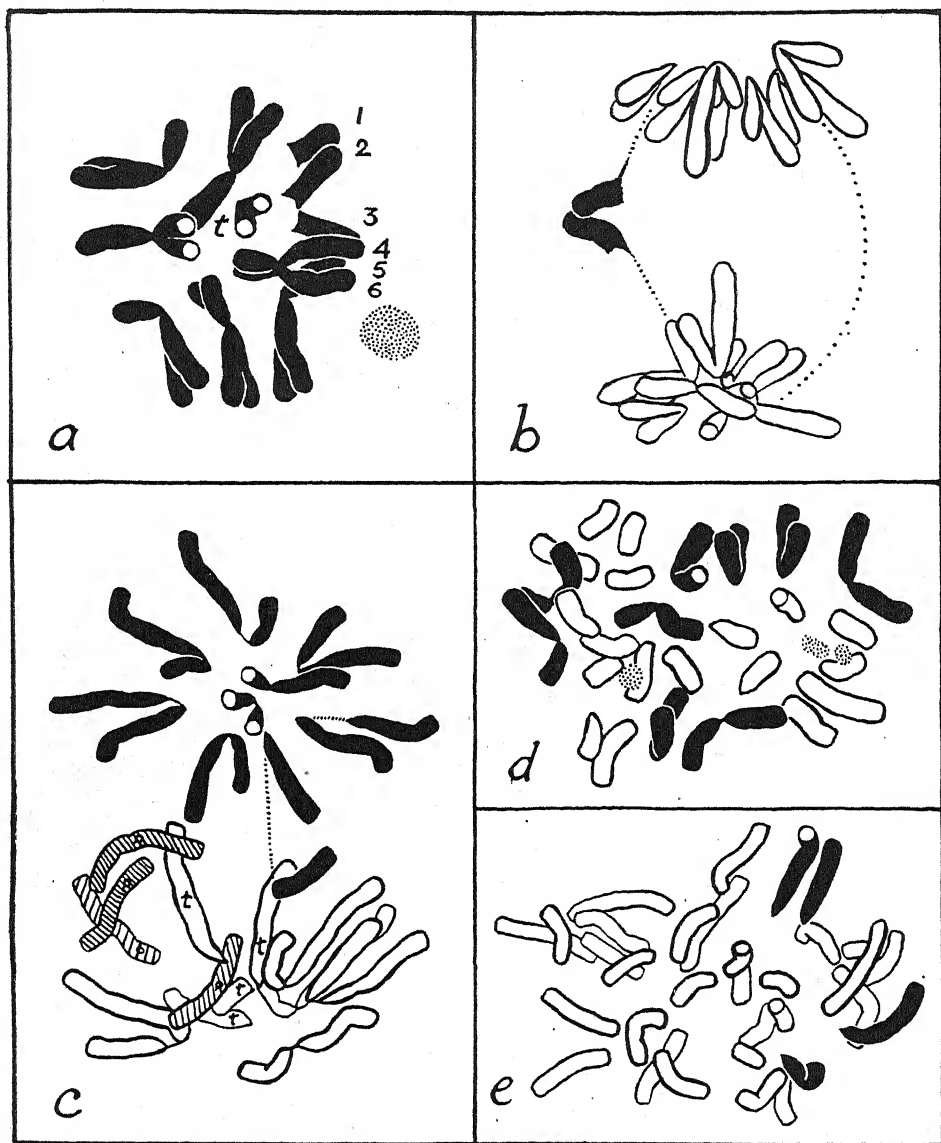
Text-fig. 8. Breakage at the centromere in *Tradescantia*. *a.* Partial breakage of two chromosomes at metaphase. *b.* Lagging of incompetent arms. *c.* Breakage at anaphase of four daughter chromosomes on one side, two on the other, into competent and incompetent arms (telo- and acentrics). *d.* 18 arms on one side, 9 chromosomes on the other. *e.* Complete breakdown, 4 telocentrics still seem to be competent. 28 incompetent. 2 BE + gentian violet. × 2600.

place before anaphase begins and give two unorientated and probably acentric chromatids and one telocentric chromosome in place of each two-armed chromosome (Text-fig. 8a). The acentrics lag at anaphase (Text-fig. 8b).

Breakage of the centromere during anaphase is likewise to one side of it, and thus gives one telocentric and one apparent acentric (Text-fig. 8c). Sometimes all the daughter chromosomes to one side of the plate are
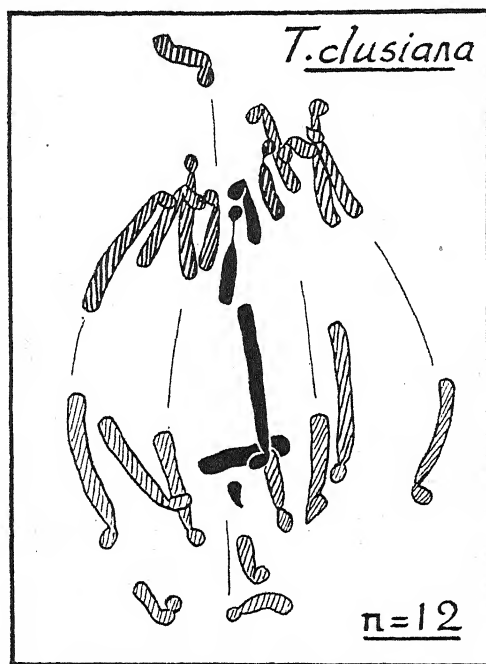
Text-fig. 9. Weakness and breakage at the centromere in anaphase of *T. Clusiana*. Unequal daughter chromosomes indicate earlier breakage of bridges.

affected (Text-fig. 8d) and sometimes the breakage is complete and the spindle is then disorganized (Text-fig. 8e).

Weakness at the centromere is not peculiar to our *Tradescantia*. It occurs also in bridge chromatids of *Tulipa Clusiana* (Text-fig. 9). Wholesale breakage also affects meiosis in *T. orphanidea*. Some of the breaks seem to be at the centromere, but the result is catastrophic and as a whole is beyond analysis. Since the species is diploid the consequences are preserved only in unreduced pollen grains (Text-fig. 10b).

Text-fig. 10. *T. orphanidea* (2x=24). *a*. Metaphase I showing 45 bodies and only 24 centromeres. *b*. Tetraploid pollen grain with partly abortive anaphase, showing pairs of centrics and dicentrics, and centric fragments, derived from breakage at meiosis. Total centromeres 92. Acetocarmine. ×1400.

3. The case of *Tulipa fragrans*

(i) *Conditional*

The incidence of breakage in this plant was exceptional and instructive. The breakage occurred in flowers of four bulbs of one clonal group from a collection of plants growing together in a cool house. It occurred in 1939 but not in 1940. It occurred in one-third of the cells (Table 3). In that third its frequency distribution was not unlike that expected and found in X-rayed samples (Newcombe, 1941). But the distinctness of the two-thirds and the non-occurrence of the affected third in 1940 equally show the action of a sharp threshold for breakage.

Table 3. *Frequencies of breaks in pollen grains of one anther of unstable* Tulipa fragrans

| Breaks = C_0	0	1	2	3	4	>4	Total
Metaphase	63	—	1*	—	—	29	93
Anaphase	23	1	—	1	4	14	43
Total	86	1	1	1	4	43	136

* $2x+1$ pollen grain.

How this threshold works appears from the character of the pollen produced. The affected plant had 54 % of good pollen as against 65 % in a normal plant in 1939. The slightness of the difference indicates that breakage in a third of the cells was a consequence of bad development rather than a cause of it. This assumption is supported by the sizes of the chromosomes, which are widely variable in normal cells of the abnormal plant (Text-fig. 11). Depressed conditions can seemingly reduce chromosome size in a less specific way than the genetic mutations already known (Darlington, 1937 a).

(ii) *Mechanical*

The classification of broken chromosomes depends on the recognition of constrictions. In *Tulipa*, as in *Tradescantia* and *Trillium*, there are no regular nucleolar organizers and in consequence no regular nucleolar constrictions. Nucleoli are attached to chromosomes terminally, if at all, and trabants, though sometimes seen in root-tips, are not found at all in pollen grain mitoses. All constrictions are therefore centric, and the classification of polycentric chromosomes is simple and accurate.

A second difficulty that might arise is that of distinguishing centrics from acentrics at metaphase when they are very small. In every cell however the positions of the twelve centromeres (or 25 in Text-fig. 12B) have been located on not-too-small chromosomes at metaphase, so that

the difficulty has not arisen. At anaphase on the other hand the distinction is simple. Small centric chromosomes have been found only twice. They move to the poles in company with their larger fellows (Text-fig. 14 D) or even in advance of them (Text-fig. 14 B). Small and large acentrics equally lag behind.
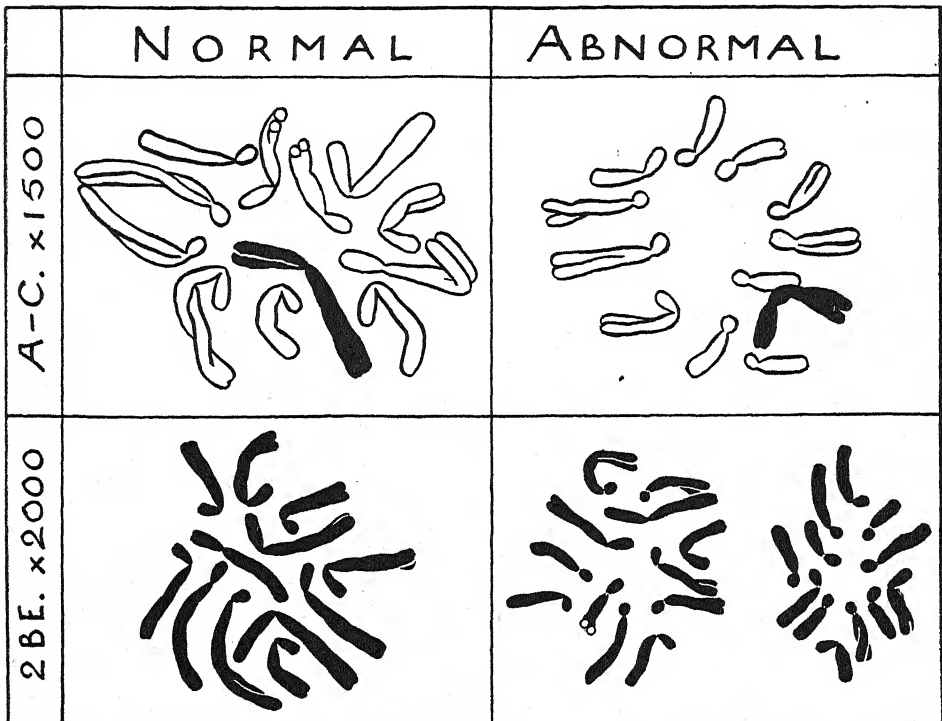
Text-fig. 11. Pollen-grain metaphases in *Tulipa fragrans* with different fixatives to show size differences between normal plants and extreme cells in abnormal plants (cf. Pl. 11, fig. 1).

The movements of acentric chromosomes deserve more detailed study than has yet been given to them, and these pollen grains are well suited to the purpose. We already know from what happens to acentrics following inversion crossing-over at meiosis that they are passive in relation to the spindle.[1] Following their movements can therefore show us what the spindle does to a foreign body that lacks the active reaction of a centromere.

[1] *Pace* Carlson (1938, *Proc. Nat. Ac. Sci.* **24**, 500–7) who perhaps does not realize that acentrics lack the organ which separates sister chromatids at anaphase and is also the means of holding them together until this separation occurs.

At metaphase the distance apart of two associated acentric chromatids is an inverse function of their size. In the figures this relation is sometimes distorted either by the pressure of smearing or by variation in the angle of vision. The effect however is clear enough. It depends no doubt on the position being one of equilibrium between a repulsion which is a function of surface and an attraction which is a function of mass.

Acentric chromosomes lie indifferently on or off the spindle, just, it seems, where they happen to have been spilt by the dissolving nucleus.
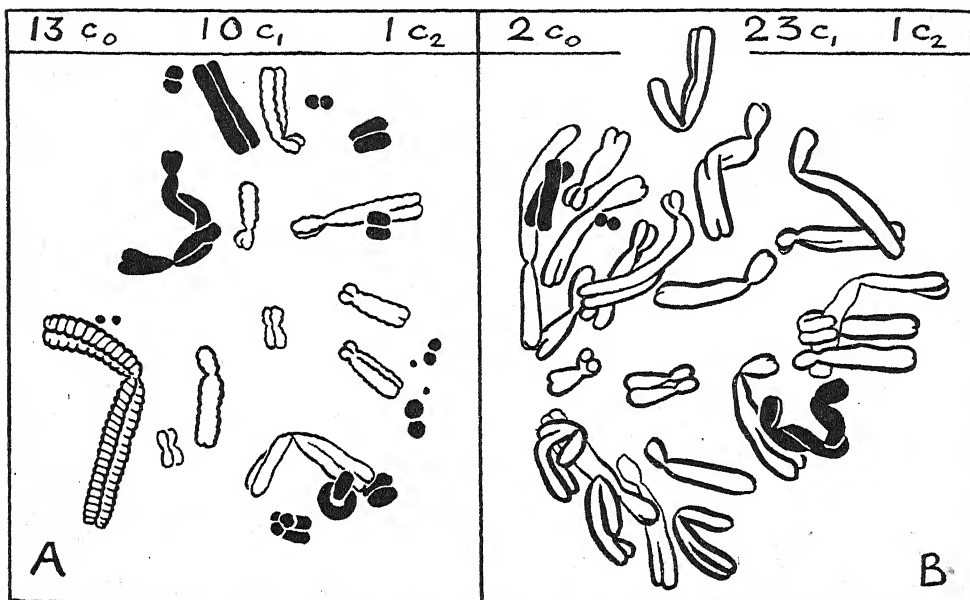
Text-fig. 12. Metaphases with monocentrics in outline, dicentrics and acentrics in solid. A. Haploid (cf. Pl. 1, fig. 4). B. $2x+1$ nucleus. Figs. 12–17, acetocarmine. $\times 1800$.

When anaphase begins they, like the centric chromosomes, begin to undergo the lapse of chromatid attraction. This does not at once lead to any regular movement, but those lying in the spindle move apart axially. Those outside the spindle often come to lie end to end, in which position they form characteristic pairs at telophase (Text-fig. 16). Acentric chromatids attached in this way to centric ones are no doubt responsible for the "achromatic lesions" invoked by Giles (1940) and others in describing chromosome breakage.

(iii) *Qualitative*

Apart from its frequency distribution, nothing at first seems to distinguish the conditions of breakage in *Tulipa fragrans* from those we

at present associate with X-ray treatment. We merely have to assume that breakage takes place during the previous resting stage and before division of the chromosomes into chromatids. All cells can then be scored for the number of acentric fragments, each fragment consisting of paired chromatids (C_0''). This number gives the number of effective breaks recorded in Table 3.

The second important datum is the number of polycentric chromosomes (C_2, C_3, etc.) which result from reunion between pairs of centric fragments, i.e. non-sister reunion (**NSR**). Their occurrence warns us that reunion must also be expected between centrics and acentrics and also between pairs of acentrics. These events are attested by some of the new chromosomes being longer than any of the unbroken complement (cf. Tables 5 and 7); these are both monocentric (Text-figs. 13 B, 17) and acentric (Text-figs. 14, 17). They are equally attested by the formation of ring chromosomes, of which both acentric (Text-fig. 12 A), and dicentric (Text-fig. 13 C) examples have been seen in this clone. The aggregate number of original breaks must therefore be much greater than the number of acentrics recorded. When restitution of old unions also is considered, the total is perhaps, as Fabergé (1940) has suggested, in treating X-ray breaks, more than five times as many.

The third important datum is the frequency of sister reunion. This is unequally distributed amongst the cells. Sister reunion can be scored certainly in centrics at anaphase, less certainly in acentrics at metaphase (Text-fig. 13 A) and anaphase (Text-fig. 15), and most uncertainly when it occurs in centrics at metaphase, where it must always be under-estimated. We assume that all single acentric bodies are due to sister reunion. There are few or none of these among the smaller acentrics.

We now turn to the three observations which seem to protest against these qualitative generalizations.

First, there is the triple fusion of separate chromatids in Text-fig. 13 B, and the tricentric chromatid in Text-fig. 15. These are the only examples we have found of non-sister reunion of chromatids, but that does not mean that such reunion is rare, for only when the two sister chromatids reunite differently will their independence be discoverable. It might be objected here that we are perhaps dealing with chromatid breakage as well as chromatid reunion, but that possibility is excluded by the lengths of what should on this view be the unbroken chromatids: those in 13 B are too short for unbroken chromatids, that in 15 too long, and therefore the result of fusion. The "chromatid breaks" described by previous authors as mixed with chromosome breaks in occasional cells may well
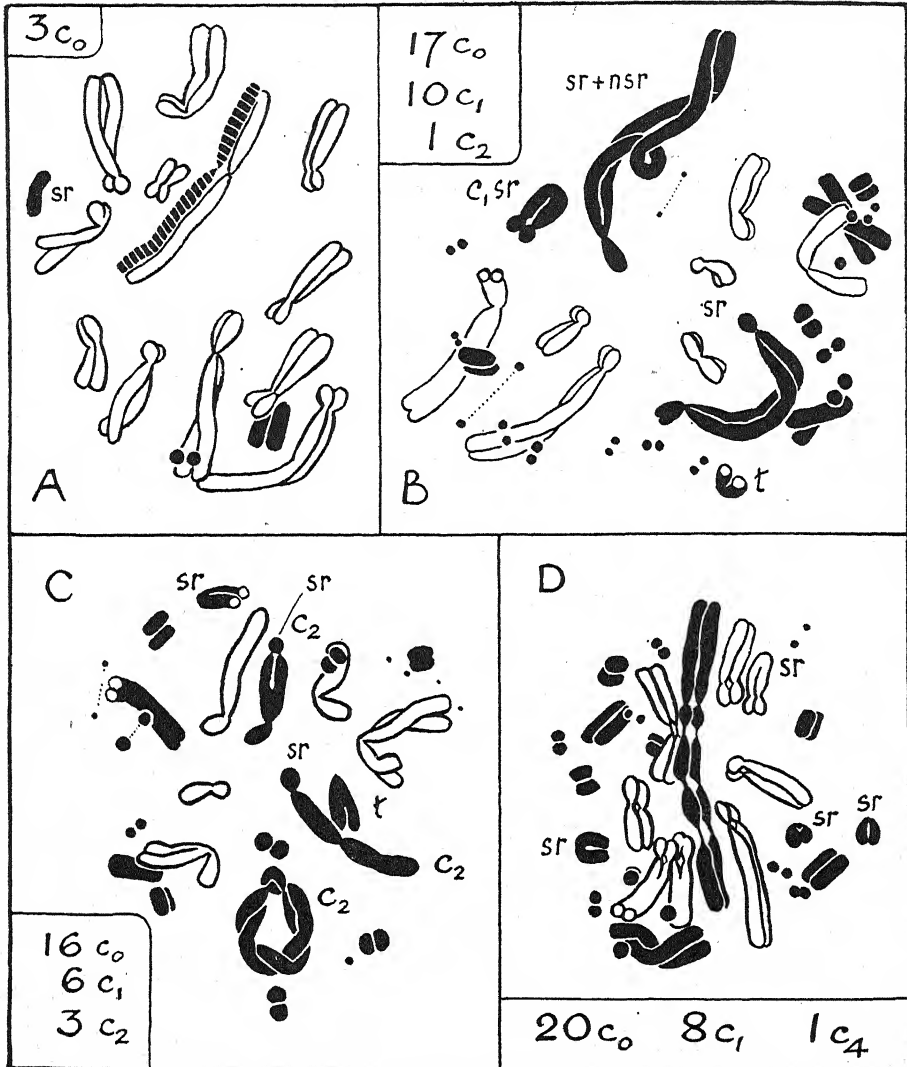
Text-fig. 13. Metaphases with chromosome breaks and reunions. C is Pl. 11, fig. 3, D is Pl. 11, fig. 2. **SR**, sister reunion of chromatids. **t** = telocentric. A, longest chromosome has 29 coils. × 1800.
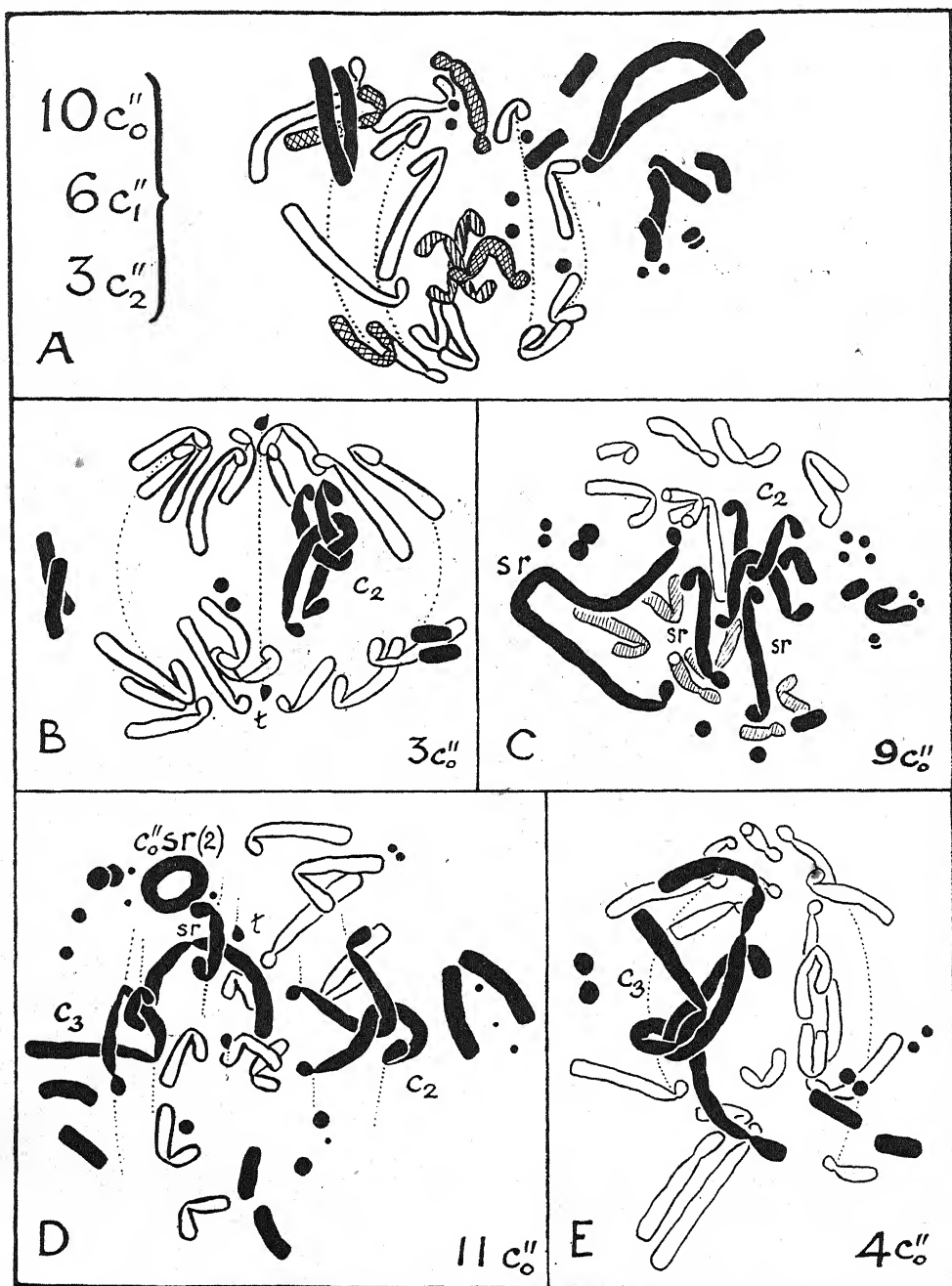
Text-fig. 14. Anaphases showing numbers and behaviour of acentrics and dicentrics in *T. fragrans*. ×1800.

have been likewise chromosome breaks which have been followed by chromatid fusions.
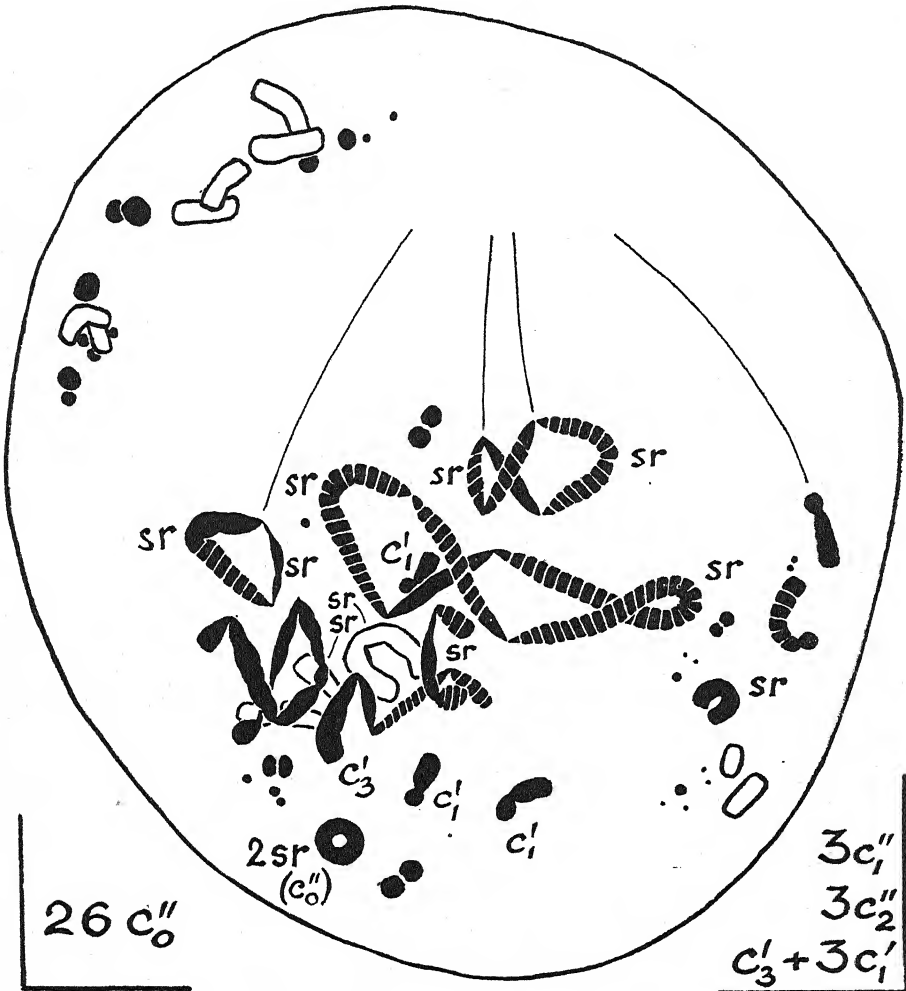
Text-fig. 15. Anaphase showing maximum of breakage and of sister and non-sister reunion of chromatids. Acetocarmine. ×2200.

Secondly, there are two examples of what seem to be single acentric rings; neither of these however can be single chromatids (Text-figs. 14D, 15). Two possible interpretations are shown in Text-fig. 19. The double acentric ring chromatids are interlocked and the dicentric ring is coiled several times. Acentric rings could thus have had a half-coil to give

continuous chromatids at metaphase, which with the lapse of attraction at anaphase could have simply uncoiled. We have seen monocentric rings of this type in various tulips (Upcott, 1937*b*), and they have also been illustrated by McClintock (1938*a*). Alternatively these anaphase
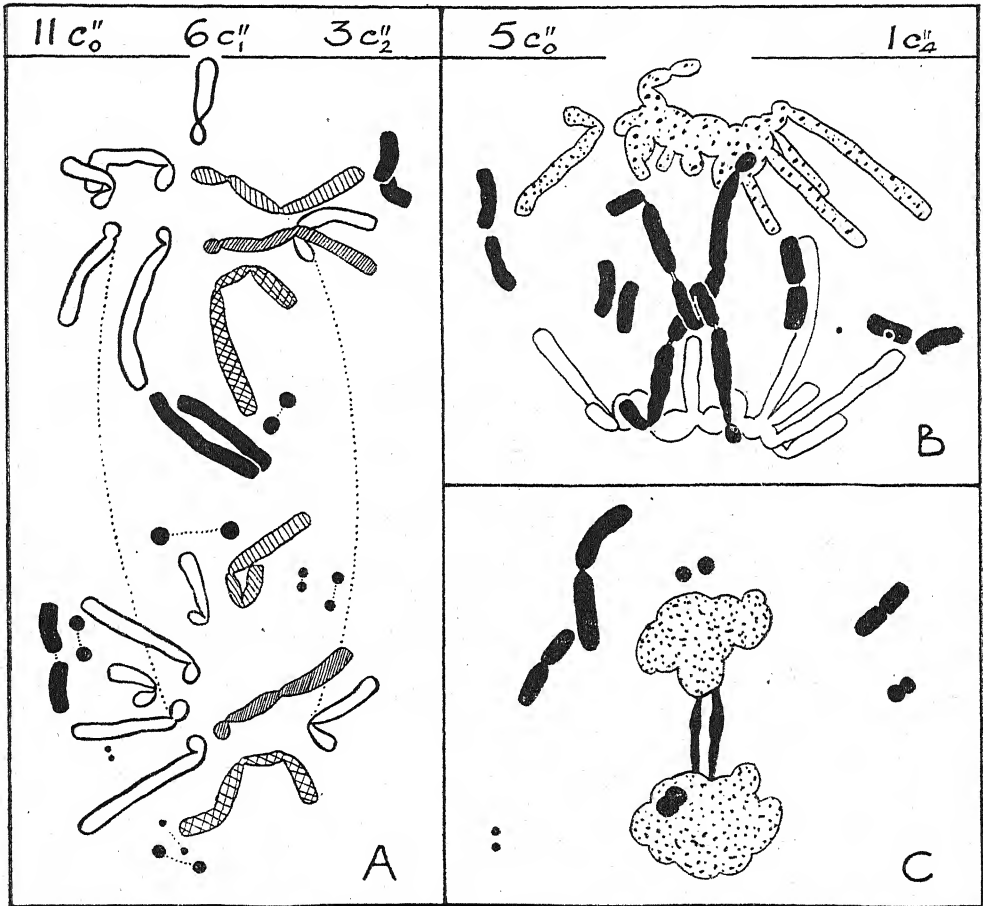
Text-fig. 16. Anaphase and telophase. ×1800.

rings could be pairs of deleted chromatids with sister reunion at both ends. Since sister reunion occurs elsewhere in the same cells this is the view we have provisionally adopted.

(iv) *Quantitative*

The broken chromosomes of *Tulipa fragrans* show several properties which require statistical description. The qualitative description that we
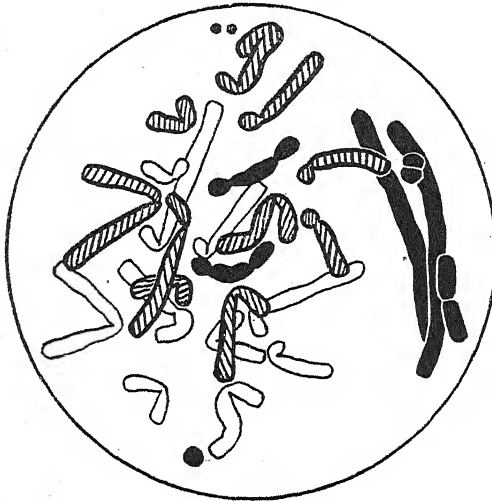
Text-fig. 17.　Large acentrics and small dicentrics derived presumably from asymmetrical chromosome interchange. × 1800.
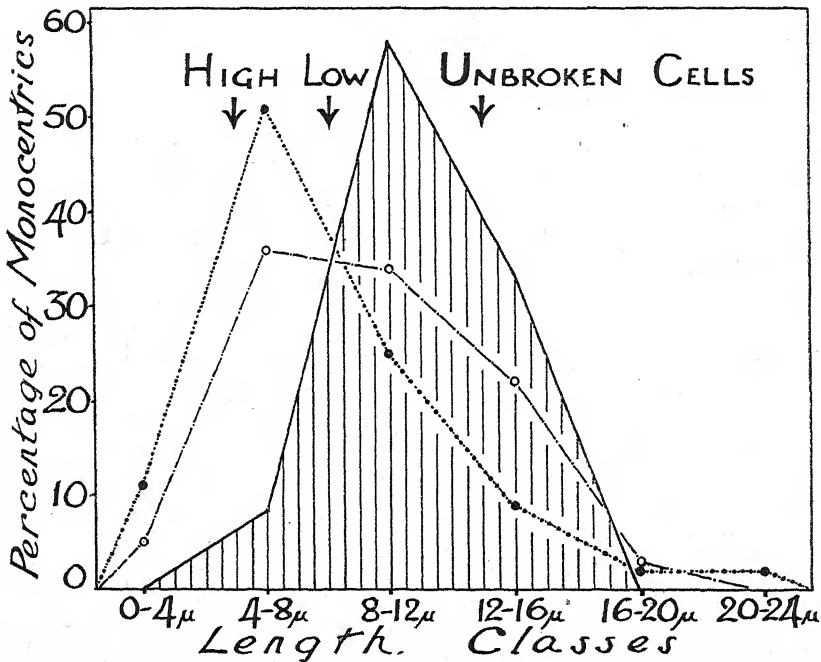
Text-fig. 18.　Graph showing lengths of monocentric chromosomes, (i) in unbroken cells, (ii) in cells with low breakage (Figs. 12 A–12 B) and (iii) in cells with high breakage (Figs. 15–13 C). Data taken from Table 7 but classified in larger groups. Note that as the mean length falls the maximum length rises owing to chance reunion. This shows that the natural variation in length is not a chance one.

have so far given makes this possible. Hitherto the direct analysis of chromosome breakage has had limited objectives. It has therefore been piecemeal in design. Sax (1940) has largely made use of dicentrics and rings which can be taken as indications of reunion. Fabergé (1940) has confined himself to total bodies which are compounded of the acentrics representing net breakage less the polycentrics representing net fusion.[1] Most workers have failed to distinguish in practice between sister and non-sister reunion and none of them have considered the intra-cell and inter-cell differences of even their gross estimates and their partial and inconsistent classifications. A few have made surprising discoveries without the comparison of untreated material. In a word generalization has preceded analysis. Valuable results have been reached by, or in spite of, these methods. But they have made it plain that what we need now is a detailed analysis of a small sample both for spontaneous and for induced changes.

The sample recorded from one slide in Table 3 showed the breakage as a threshold reaction. Another sample including only the broken cells reveals other properties, first in regard to the conditions of reunion, secondly in regard to the real or apparent positions of breakage.

The distribution of reunions can be considered as between the different chromosome types and as between different cells. For both purposes we can assume that breakage is a condition of new unions of both sister and non-sister types. And acentrics are a measure of breakage. As between types produced with and without non-sister reunion we can consider the chances of sister reunion of other ends.

Table 4 shows the result. When allowance is made for most of the monocentrics being unbroken or restituted chromosomes the observations seem to agree with an equal chance of sister reunion for the broken ends of monocentric and polycentric chromosomes. The acentrics on the other hand have far too little sister reunion, and this is due to its absence in the very small acentrics, an observation which we shall recall later.

As between cells, non-sister reunion (**NSR**), shown by polycentrics and rings, shows a near proportionality with the number of breaks shown by acentrics. This net reunion does not quite keep pace with net breakage, and for an obvious reason: the recognition of reunion depends, in poly-

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[1] Fabergé has found five cells with $n-1$ bodies (i.e. with dicentrics and invisible acentrics) in four preparations together with 123 cells with n bodies. Since the numbers of dicentrics in these was not recorded, it is impossible to estimate what proportion of acentrics in all cells ($n+1$, $n+2$, ...) were invisible and hence the degree of accuracy of the recorded data.

centrics, on the absence of a second breakage between the point of reunion and a centromere or, in rings, on its total absence.

This principle inherently applies to Sax's fractionation tests. He finds (1940, fig. 5) that four doses when widely separated fail to give four times as many non-sister reunions (judged from polycentrics and rings) as does one dose multiplied by four. Repetition of doses beyond a low value begins to break the evidence of the reunions produced by a single

Table 4. *List of net observable chromosome changes in*
Tulipa fragrans *pollen grains*

Fig.	$C_0 = B$	C_1	C_2	C_3	C_4	SR	NSR
15	26 (4) r′	3 (3)	3 (5)	1*	—	*12*	*6*
13 D	20 (3)	8 (1)	—	—	1	*4*	*3*
13 B	17	10 (2)*	1 (1)	—	—	*3*	*1*
13 C	16 (1)	6	3 (2) (r″)	—	—	*3*	*4*
12 A	13 r″	10	1	—	—	*0*	*2*
16 A	11	6	3	—	—	*0*	*3*
14 D	11 (2) r′	7 (1)	1	1	—	*3*	*4*
14 A	10	6	3	—	—	*0*	*3*
14 C	9	10 (3)	1	—	—	*3*	*1*
16 B	5	8	—	—	1	*0*	*3*
17	5	10	1	—	—	*0*	*1*
14 E	4	9	—	1	—	*0*	*2*
14 B	3 (1)	10	1	—	—	*0*	*1*
13 A	3	12	—	—	—	*1*	*0*
12 B	2	23	1	—	—	*0*	*1*
Total 15 cells	155 (11)	138 (10)	19 (8)	3	2	29	35

r″ (chromosome ring) = NSR″. SR in brackets.
* 2 NSR′.

Table 5. *Frequencies of length classes (in microns) of different*
chromosome types (cf. Text-fig. 18 and Tables 6–8)

Classes ...	0–4	4–8	8–12	12–16	16–20	20–24	24–28	28–32	32–36	Total
C_0	134	16	3	—	2	—	—	—	—	155
C_1	10	55	39	21	3	1	—	—	—	129
[Unbroken	—	*1*	*7*	*4*	—	—	—	—	—	*12*]
C_2	—	1	6	8	3	—	—	1	—	19
C_3	—	—	1	—	—	1	—	1	1	3
C_4	—	—	—	—	1	1	—	—	—	2

dose with incomplete reunion. There must always be a correction for multiplying and the optimum frequency of breaks for the production of dicentrics is not the maximum frequency. Or more generally speaking, the empirical combination from which breakage and reunion are inferred can show no linear relationship with either component.

Of the two types of non-sister reunion there are one centric ring union and thirty-one polycentric non-ring unions. This agrees well with the expectation of random reunion of arms as between combinations, internal and external to the chromosome, of 1 : 22, where n is 12.

Again, as between cells, sister reunion, unlike non-sister reunion, is disproportionately frequent in cells with much breakage.

We can now turn to the positions of breakage. When the sizes of acentric fragments are considered, three of their properties at once leap to the eye. First, they are smaller than random breakage allows. Secondly, they are more uniform within cells, particularly within those with most breakages, than this same randomness would allow. Thirdly they do not show sister reunion. They are rarely, if ever, single.

To determine the soundness of this impression we have measured all acentrics (Tables 5, 6 and 7). This approximation is accurate enough for the large-scale relationships which alone are significant. It will serve as a basis of comparison not only with future X-ray observations but also with ideal expectations. We can ignore refusion since centrics and acentrics are shown by polycentrics on the one hand, and by very long acentrics on the other, to be equally fusible. We may then argue that telocentric chromosomes with an equal chance of breakage in all parts will give centrics and acentrics of equal length. Equal-armed chromosomes under the same conditions will give the maximum disparity of 3 : 1. Yet the tables show that the average length of the centric chromosomes ($9.77\,\mu$) is more than four times that of the acentrics, while the monocentrics alone are scarcely less than that.

Table 6. *Lengths in microns of chromosomes of different centric valencies in fifteen cells recorded in Table 4*

Class	C_0	C_1	C_2	C_3	C_4	Total C_{1-4}	Total C_{0-4}
Number	155	138	19	3	2	162	317
Length	346·5	1228	267·5	65·5	50	1611	1957·5
Mean per chromosome	2·23	8·9	14·1	21·8	25·0	9·77	—
Mean per C_1	—	8·9	7·0	7·3	6·3	8·3	10·1

Acentric chromosomes are therefore derived (i) from single breaks too near the end or (ii) from double breaks too close together. The first of these explanations would be opposed to that reached by Sax & Mather (1939) from X-rayed cells and by Giles (1940) from spontaneous changes in *Tradescantia*, where the breaks are too near the centromere. The frequency of breaks per cell was much lower in these two samples, however. The second explanation is favoured by the fact that the low average length depends on a distinct group of small acentrics.

The special position of small acentrics is shown by a complete record of their sizes (Table 7). For an analysis of these we are indebted to Dr Mather. He finds that although the mean lengths of acentrics are not significantly different in individual cells, nevertheless in groups of cells

Table 7. *Statistics of size and frequency of acentric chromosome fragments in Tulipa fragrans pollen grains (chromosome breaks)*

Fig.	Lengths of C_0 in μ												n	$L(\mu)$	Mean	V_x
	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	16-17	19-20				
15	11	5	3	4	1	2	—	—	—	—	—	—	26	50·0	*1·9*	2·65
13D	7	7	3	3	—	—	—	—	—	—	—	—	20	37·0	*1·9*	2·87
13B	12	3	1	—	1	—	—	—	—	—	—	—	17	20·5	*1·2*	2·97
13C	5	6	2	1	1	—	—	1	—	—	—	—	16	33·0	*2·1*	4·26
12A	4	4	3	1	—	—	—	1	—	—	—	—	13	26·5	*2·0*	3·60
16A	5	3	2	1	—	—	—	—	—	—	—	—	11	21·5	*2·0*	0·87
14D	5	2	—	—	2	—	—	1	1	—	—	—	11	28·5	*2·6*	7·09
14A	3	—	—	—	2	—	1	—	—	1	1	1	10	43·0	*4·3*	26·40
14C	5	2	—	—	—	1	1	—	—	—	—	—	9	13·5	*1·5*	2·75
16B	1	—	—	—	—	—	—	—	—	—	1	—	5	14·5	*2·9*	1·80
17	1	2	1	4	1	—	1	—	—	—	—	1	5	26·5	*5·3*	64·20
14E	1	1	—	1	—	—	—	—	—	—	—	—	4	9·0	*2·3*	2·92
14B	1	1	—	1	—	—	1	—	—	—	—	—	3	11·5	*3·8*	6·33
13A	1	1	—	—	—	—	—	—	—	—	—	—	3	5·5	*1·8*	2·33
12B	1	—	—	—	—	1	—	—	—	—	—	—	2	6·0	*3·0*	12·50
Total	62	39	15	16	5	5	3	4	1	2	1	1	155	346·5	*2·23*	7·21

Mean lengths of 79 acentrics in 4 high cells: 1·78 μ.

Mean lengths of 76 acentrics in 11 low cells: 2·82 μ.

they are different. Those with most acentrics have shorter acentrics just as they have shorter centrics. The very small acentrics increase disproportionately with the total number just as they do in X-ray experiments. In consequence the variance falls significantly as the total number of acentrics per cell rises, and at its lowest value is too low to be considered as a random sample from the same population as the highest values.

How then do we account for an excess of close breaks? Such a group might be taken by themselves and assumed to be in a two-break class. They correspond in size with Muller's minute rearrangements and the minute deletions of Demerec *et al.* (1938), and we may recall that Sax (1938) and Muller (1940) attributed them to the occurrence of two close breaks from the spread of a single ionization; in other words, two close and related breaks. Such a view might account for the specially high frequency of small acentrics in X-rayed material, but it is ruled out in the present instance by spontaneity. As we saw, similarities of X-rayed and natural breakage are likely to be due to similarities of reunion, not of breakage. It is ruled out also by observations of X-rayed *Tradescantia*. The frequencies of very small acentrics seem to increase with the square of the dosage, and thus indicate a two-hit, or at least a complex, origin for these fragments (Newcombe, unpublished; cf. Sax, 1940).

The solution of the riddle is provided by the absence of sister reunion amongst small acentrics. Newcombe finds the same property in X-rayed *Tradescantia*. This would be expected if each chromatid were a ring, non-sister reunion of pairs of breaks having anticipated and prevented sister reunion of separate breaks. With close breaks non-sister reunion will have an exceptional advantage, not only against sister reunion but also against restitution (Muller, 1940, footnote, p. 39). It may be therefore that spontaneously as well as from external impact we have an excess of minute fragments owing to non-restitution of those pairs of breaks which happen to occur close together.

An indirect method of testing this view arises from the frequency of sister reunion. The proportion of all reunions which are sister reunions increases with the frequency of acentrics. Yet the proportion of reunion as measured by the Empirical Coefficient does not significantly vary (Table 8). This relationship would arise if the potentiality for sister reunion and the susceptibility to breakage both increased as the time of split approached. The first of these suppositions is inevitable, since the shorter the time for non-sister reunion the more ends there will be free for sister reunion. The second is *a priori* likely, since at meiosis the moment of reproduction is the moment of greatest weakness. The occurrence of

chromatid reunions from chromosome breakage as well as of sister reunions on a scale not previously observed strengthens this view. And finally the rarity of post-split reunions seems to clinch the argument.

Table 8. *Grouped data showing breakage and reunion frequencies and the empirical coefficient of reunion* ($ECR = (SR + 2NSR)/2C_0$) (*from Table 4*)

Cells by C_0	C_0	SR	NSR	SR/C_0	NSR/C_0	ECR
26	26	12	6	*0·46*	0·23	**0·46**
20–16	53	10	8	*0·19*	0·15	**0·24**
13–9	54	6	13	*0·11*	0·24	**0·30**
5–2	22	1	8	*0·05*	0·36	**0·39**
Total	155	29	35	*0·19*	0·23	**0·32**

4. ELEMENTS OF STRUCTURAL CHANGE

(i) *The time of split and sister reunion*

The time of split and its effect on reunion, as we must now realize, lie at the root of the interpretation of the effects of X-ray breakage. Since the injuries of *Tulipa fragrans* show (apart from the threshold) every analytical similarity with induced breakage and also provide a synthetic picture which is not otherwise available, we must try to use them in attacking this problem of interpretation.

All the breaks found in *T. fragrans* are consistent with their having taken place before the chromosomes divided. This is not surprising since Newcombe (unpublished) finds that the chromosomes divide very late in the pollen grain cycle in *Tulipa*. Similarly, all the reunions of non-homologous parts are double reunions, i.e. of chromosomes not of chromatids, except three (Text-figs. 13*b*, 15, 20). Sister reunions of chromatids (**SR**) on the other hand are found in all types of broken chromosomes but the smallest. This corresponds with the earlier findings, that the chromatids which are formed by division after breakage can reunite with themselves or fail to divide at the end. It also corresponds with the described origin of iso-chromosomes (Darlington, 1940).

On this view we must look at the broken end of an unsplit chromosome as having five possibilities before it: (i) restitution, (ii) non-sister reunion of the whole chromosome, (iii) sister reunion of its two chromatids, (iv) non-sister reunion of one or both of its chromatids separately and (v) non-reunion (Text-fig. 21). We should expect that restitution would take place at a rapidly decreasing rate as time after breakage proceeds. Further, non-sister reunion should begin after restitution has begun and should then limit the frequency of restitution in proportion to the concentration of breaks in the vicinity, by a process of *competition*.

Text-fig. 19. Types of structure found with sister reunion (SR) and non-sister reunion (NSR); acentric rings can arise in either way. Dicentric chromosomes (C_2'') require NSR; dicentric rings, 2 NSR, dicentric loops SR and NSR independently. From Text-figs. 6c, 13 B, C, 15.

Text-fig. 20. Consequences of different combinations of chromatid reunions after chromosome breakage in *Tulipa fragrans*. (1) In Text-fig. 14 D, (2) in Text-fig. 15, (3) in Text-fig. 13 B.

How far such competition may occur is indicated by our records, in this way. Whatever reunion takes place in the resting nucleus demands movement, but very little movement can actually occur. The resting condition of the pollen grain nucleus has just been signally demonstrated by the retention of chiasma relationships through a whole resting stage
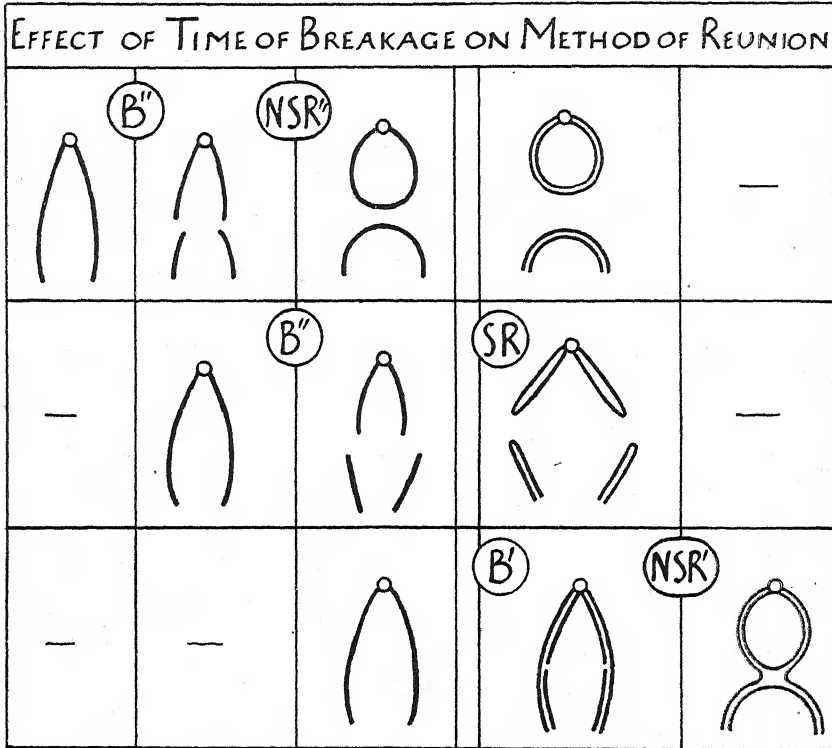
Text-fig. 21. Possible time seriation of break to give three possible types of reunion under conditions of rapid reunion. With slower reunion the earlier breaks will be capable of giving the later types of reunion and the middle type will be able to give either the earlier or the later type. Types of non-reunion and differences in position of the two breaks omitted.

in *Fritillaria* (Barber, 1940). Of course a twisted chromosome when broken in the middle will untwist and move, but its movement will be restricted. A very small proportion of original breaks (even smaller than Fabergé's first estimate) should therefore be capable of making new reunions, unless the concentration of breaks is very high.[1] Nevertheless, as we saw, the empirical coefficient is consistently almost as high as a

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[1] We understand from Dr Catcheside that he has arrived at a similar conclusion on experimental grounds.

third. Thus our acentrics must be taken as a small sample of the original breaks. This is further supported by the evidence of selection favouring the preservation of fragments from pairs of breaks close together.

When we admit that restitution is an important component of the potential behaviour of each break, the principle of competition follows inevitably and its conditions become an object of enquiry. We know that the higher the dose the greater the number of breaks per nucleus. The greater this number the greater will be the chance of new union of each of them as opposed to restitution. But this increased chance will depend on all the breaks being present in the nucleus at once. That is, they must occur close enough in time as well as in space to compete with restitution. Thus a high dose will give a disproportionate or exponential frequency of observable new unions, not owing to two breaks being necessary for each new union but owing to a much larger number being necessary for two of them to be effective (Text-fig. 22). That the exponent is not higher than two is due to higher doses, as we saw, undoing a larger proportion of their own observable effects. Similarly, the fractionation of a dose in time will give an advantage to restitution. A limiting effect in separation of the split doses will be reached when the vast proportion of possible restitutions has occurred.

The effect of timing of separate breaks as shown by experiments with intensity and fractionation of dosage are of vital importance in showing the relative behaviour in rejoining of old and new combinations of ends. Sax's data (1940) seem to show that fractionation reduces the number of new rejoins. Fabergé's data (1940) seem to show that it reduces the number of net breaks, i.e. total breaks minus total rejoins. These records are compatible with one another on the assumptions common to both workers (i) that total breaks are directly proportional to dosage and (ii) that a proportion of the breaks undergo restitution. They require however that this proportion differs with intensity or fractionation in the way we assume in Text-fig. 22.

Thus competition introduces a whole series of disproportions between dosage and effect in its temporal relationships. The small fragments show the same disproportions in its spatial effects. On this view it is not necessary to suppose, as Sax (1939) has done, that: "If the radiation is given slowly the first break may heal[1] before a second break occurs in an adjacent chromosome", or (1940) that "broken ends of chromosomes may remain in an unstable condition[1] for as long as an hour before fusion" (and no more) but rather that during the first hour after breakage the

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[1] Cf. note on ambiguous usages in Appendix.

proportion of breaks which are lost by restitution depends on the competition for reunion amongst broken ends, including sister ends at the time of splitting. There is indeed no present evidence that a broken chromosome end ever heals against non-sister reunion. The only occasion in later life when two broken ends are likely to find themselves in contact is at pachytene when, as we shall see, evidence of non-sister reunion of ends is to be found in bridges without fragments at meiosis. The gradual
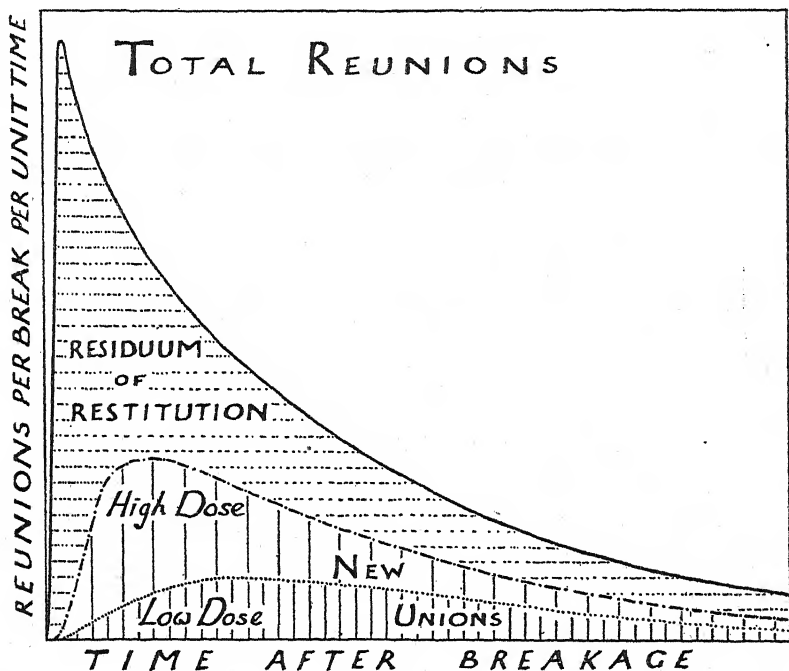

Text-fig. 22. Graph illustrating the consequences of a hypothesis of competition to be derived from the occurrence of a restitution of breaks. The situation is simplified by assuming (i) simultaneity of all breaks and (ii) equality of total reunions per break for all doses.

decrease of the proportion of new rejoining as the stage of X-raying advances (Sax, 1938, Table 3) agrees with this view of permanent ability to rejoin.

Entirely different is the case with sister reunion. McClintock and Muller and we ourselves have shown that sister reunion of broken ends is a capacity which is conditioned by the cell. It may be stopped at once or continued for ever. The issue between healing and non-healing has been confused by failure to distinguish in observation or in theory between the

processes of non-sister and sister reunion the one of which requires movement and fusion while the other does not.

Two apparent discrepancies can now be resolved. Sax (1938) finds sister reunion in cells containing chromatid reunions, or, as he says, chromatid breakages, only. But if we suppose that ends do not heal and that sister reunions occur after chromosome breakage during an intermediate period when chromosome reunion is no longer possible, this
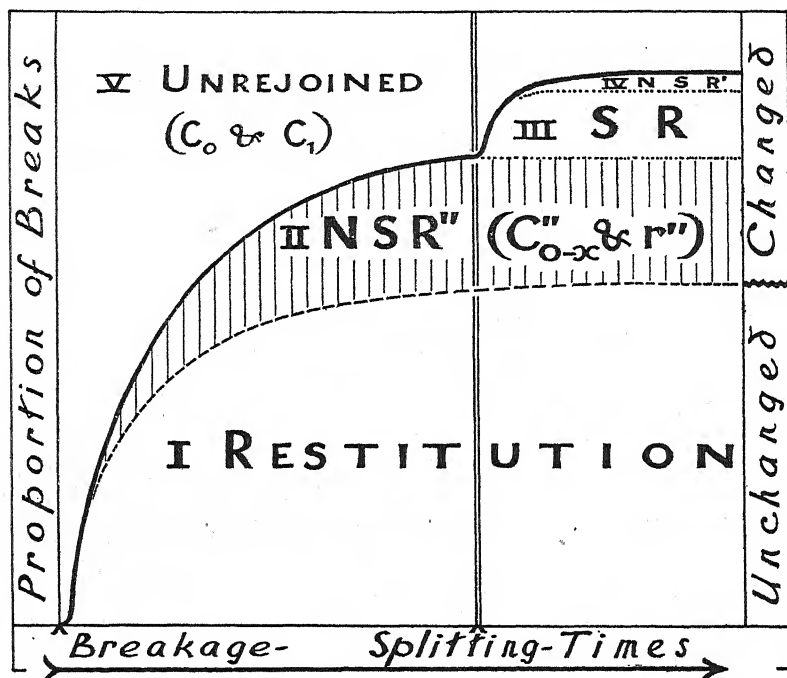
Text-fig. 23. Graph showing the integrated effects of breakage before splitting as in *Tulipa* on the principle used in Text-fig. 22, combined with the occurrence of sister reunion (**SR**) after splitting. The *Tulipa* sample consists of a mixture of cells and chromosomes with different times of breakage and therefore different proportional developments of the four superimposed curves.

difficulty disappears and his observations are consistent with ours (Text-fig. 21).

Secondly, to account for differences in healing against sister reunion of natural and induced breaks, Sax (1940, p. 56) following McClintock (1938b) invokes differences in the time of split. His argument seems to be based on two assumptions: The first is that two sister chromatids can be broken at sister points after splitting by a single hit. This assumption is held to be untenable on purely physical grounds (Delbrück, 1940). It

conflicts with our finding of sister reunion from chromosome breakage. And further, it is unnecessary on our view of restitution and delayed reunion. The second is that the difference in healing must depend on a difference in internal conditions, i.e. of split. This assumption likewise is rendered unnecessary by McClintock's own evidence of external conditioning. The time of split is always the same—the resting stage. The conditions of reunion are what change.

In the past, metaphase structures have been regarded as evidence of the state of the chromosome at the time of *breakage*. We now see that the time of *reunion* is even more important. And since on our view the time between breakage and reunion may be extended to varying degrees in different cells and organisms, the interpretation of the time of split must take account of these variations. It seems that we must distinguish between three overlapping stages of chromosome development (Text-fig. 21): those with chromosome reunion, period I; those with sister chromatid reunion, period II; those with only non-sister chromatid reunion, period III.

Accordingly, all accounts of the time of split judged from X-ray breakage must be modified in the following way. Period III will extend to the total time in which the chromosomes are split plus the time after which a perceptible number of broken ends can continue to rejoin. The earliest possible time of split is the latest time at which breakage can be followed by chromosome reunion. A long period with mixed cells (with **NSR″** and **NSR′**) means a long period during which reunion is taking place. Rarity of mixed cells can be due either to rapid reunion or to obligatory sister reunion of all unjoined breakages at the time of splitting. Healing against sister reunion is, we know, easier in some cells or organisms than others. Apparent diversity in the mechanism of X-ray breakage is therefore due to diversity in the spatial relationships and in the potentialities for union of broken ends. Real diversity in breakage arises only in its spontaneous occurrence.

Thus Mather's estimate of the time of split (1937) is an *earliest* estimate. In *Tradescantia* this estimate is perhaps only a few hours too early. In *Allium* it may be much too early. All but a minute fraction of genuine mixed breakage in the period of mixed cells (**NSR″** and **NSR′**) must be taken as corresponding to chromosome, not chromatid, breakage. In a word the chromosomes divide later in the nuclear cycle than has been supposed.

This principle has the same simplifying effect as Muller's genetical interpretation (1940) of the special consequences of delayed reunion in

Drosophila. Here, as in Maize, broken ends will undergo sister reunion to form bridges which break again after each successive division of the chromosome. Hence X-raying the sperm sometimes gives the appearance of chromatid or even half-chromatid breakage, although the chromosomes in the sperm are never divided at the time of breakage.

A third apparent discrepancy is the inversion in order of chromosome and chromatid breaks in White's production of diplochromosomes. The explanation by an inversion in the order of cell divisions (Darlington, 1937 a) has recently been confirmed by Barber's experiments.

In time of split therefore uniformity succeeds diversity: and this is true of all the various methods of enquiry. Direct observation of structure, comparison of movement at mitosis and at meiosis in diploids and polyploids, comparison of the critical consequences of crossing-over in the cell and in the progeny, consideration of the nucleic acid cycle, analysis of induced and spontaneous breakage and reunion, all these evidences combine to show, first, that the chromosomes enter the resting nucleus undivided and, secondly, that they do not divide until the next prophase is approaching or, in meiosis, until it has actually begun.

(ii) *The uses of diversity*

From its earliest definition there has been a widespread although usually implicit assumption that mutation, having a certain uniformity of results, has also a certain uniformity of causation. In the absence of contrary evidence the argument from analogy was, to be sure, a proper expedient. This argument has recently been tested by four kinds of comparison, chiefly affecting structural change in the chromosomes:

(i) One organism or tissue against another, e.g. *Drosophila* sperm against *Tradescantia* pollen grains.

(ii) One part of a chromosome against another, e.g. heterochromatin against euchromatin.

(iii) One type of change against another, e.g. gene mutation against structural change.

(iv) One method of causation against another, e.g. spontaneous against X-ray or ultra-violet mutation.

The diversity revealed may be summarized as follows:

(i) The compactness of the sperm prevents movement and therefore delays reunion in new combinations until after the sperm has entered the egg. Temperature effects and differences from concentration of dosage such as Sax (1940) and Fabergé (1940) found in pollen are not therefore found in the sperm (Muller, 1940). The pollen-tube nucleus seems to be, as

it should be, intermediate (Newcombe, unpublished). The differences found between the effects of X-raying pollen and sperm imply a difference both between organisms and tissues. The permanent fusibility of sister broken ends in *Drosophila* (Muller, 1940) and in *Zea* endosperm (McClintock, 1939), as opposed to their healing in most plant tissues, implies the same difference. Healing of sister ends is variable and is both internally and externally conditioned. The union of unbroken sister ends at mitosis and meiosis provides the extreme example of this conditioning.

(ii) The differences in breakability and indeed in general mutability in heterochromatin and euchromatin imply differences between chromosomes and also between organisms (Kaufmann, 1939; Muller, 1940).

(iii) The absence of a temperature coefficient and the direct proportionality to dosage of gene mutation (Timoféeff-Ressovsky, 1937) implies a simplicity in the effect which is not true of structural change (Sax, 1940; Fabergé, 1940). This agrees with the view that a necessary part of viable structural change, viz. reunion, is physically independent of the original breakage.

(iv) The same distinction arises from mutation-producing ultraviolet being ineffective, while X-rays are effective, in causing structural change (Muller & Mackenzie, 1939; Delbrück, 1940).

A great diversity therefore appears in the mechanisms of induced structural change in chromosomes. In most respects it is a diversity of capacity of union and reunion rather than of breakage.

The spontaneous changes, on the other hand, vary in reunion chiefly subject to the conditioning of sister reunion already discussed. In breakage they present a picture of diversity which includes the X-ray type as one of its several forms. These forms we now see can be classified according to the stage of mitosis to which each·is inherently confined, as follows:

> Resting stage: radiation breakage and reunion.
> Prophase: knot formation.
> Metaphase: centromere breakdown.
> Anaphase: chromatid sticking and breakage.
> Telophase: bridge breakage.

Thus in our present observations of spontaneous change the method and capacity of breakage itself seems to become diversified. We are in fact replacing a precise physical implement by a whole series of variable biological conditions of all degrees of precision (Table 9). Some are environmental, others genotypic. Others again, as in our *Tulipa fragrans*,

are both externally and internally conditioned. Some act at one stage of mitosis, some at another. Some act at one stage of development, some at all stages.

Table 9. *Order and kind of spontaneous chromosome breakages and unions here described in pollen*

		Change	Result	Plant or place
		I. Gene string		
A.	At meiosis	Prophase breakage	Breakdown	*Tulipa orphanidea*
		Bridge breakage	Deletion, C_0	All inversions
		Non-disjunction of bridge	C_2	All triploids
B.	From meiosis	Sister reunion	Loop chromatids	All broken bridges
C.	After meiosis			
	(i) Before split	Chromosome breakage and reunion	C''_{0-4} rods and rings	*Tulipa fragrans* 2x
	(ii) Before and after split	Chromosome breakage and sister reunion	Loop chromatids	,, ,, ,,
	(iii) After split	Sister union of true ends	,, ,,	Tradescantia 3x Hyacinthus 2x
D.	At pollen grain anaphase	Breakage of all chromatids	C_0 and C_1	*Tulipa silvestris*
		II. Centromere		
A.	At meiosis	Misdivision	$C_1 t \rightarrow iso$	*Fritillaria* 2x, 3x *Tulipa praecox* 3x
C.	After meiosis	Centric breakage	,, ,,	*Tradescantia* 2x
D.	At pollen grain anaphase	,, ,,	,, ,,	*Tulipa Clusiana* 2x

In general the spontaneous breakage that is seen is confined to the pollen. This is partly due to the pollen being the only part of the plant where the chromosomes can be broken down without fatal results to the whole organism. But perhaps a real developmental restriction may arise on this account. For, to put the matter in another way, the existence of a pollen mitosis after meiosis puts the products of structural change on the male side through a mitotic test. The test is carried out at the minimum expense and without loss of seed. Thus specific mutability in the pollen has the adaptive advantage of producing the greatest experimental results at the least reproductive risk.

The immediate value of this diversity of spontaneous change lies in its warning us that natural change is not limited to the model type exposed to our study by X-rays. Certain changes such as breakage at the centromere can occur with a regularity which endows them with an evolutionary importance they would never be allowed by the X-ray pattern. A genuine direct fragmentation *at* the centromere and *of* the centromere has long been postulated in animals, but has seemed to conflict with probability. We now see that it can take place, and take place in a wholesale fashion.

Similarly the process of diminution at anaphase, which occurs as an adaptively localized process in the development of *Ascaris* and *Sciara*, can, we now see, take place as a special accident in plants. It could therefore have arisen in the first place by a single step.

Finally we have to consider the difficult problem of structural change at meiosis. Sorting out the consequences of crossing-over between mispaired homologous segments that are reduplicated in a homozygous individual from genuinely spontaneous structural change must always be difficult. Two examples of apparent inversion crossing-over are worth considering. In inbred stocks with an abnormal meiosis conditioned by segregation of recessives it sometimes happens that bridges and fragments are found at first anaphase (e.g. in *Secale*, Lamm, 1936; *Lathyrus*, Upcott, 1937c). Is it not perhaps more likely that such aberrations are due to new inversions accumulated in the course of development or at some specific stage of development rather than to an original inversion hybridity of each individual?

Again, as we saw, bridges without fragments in cells at meiosis in *Tradescantia* (Darlington, 1937b) and in asynaptic *Pisum* (Koller, 1938) may be due to sister reunion of true ends rather than to inversion crossing-over. And the excessive frequency of second division bridges in certain tulips (Upcott, 1937b) may likewise be ascribed to sister reunion of ends from bygone breakages instead of to a special and unexpected combination of successive cross-overs. These are merely examples of the variety and specificity of types of structural change which we must be prepared to find under different natural conditions and from which we must be prepared to deduce an organized character in natural variation.

5. SUMMARY

1. Spontaneous chromosome changes in pollen grains and tubes of *Tulipa*, *Hyacinthus* and *Tradescantia* are of four kinds:

(i) Nuclear breakage and reunion of the externally induced type.

(ii) Nuclear sister reunion or terminal non-division of broken and of unbroken chromatids.

(iii) Metaphase breakage next to centromeres.

(iv) Anaphase breakage of chromatids.

2. Each of these changes is characteristic of a particular genotype, hybrid or mutant. An environmental threshold within the normal range in a clone of *Tulipa fragrans* with type (i) changes gives a bimodal frequency of changes with the following properties:

(i) Chromosome breaks are followed by chromosome reunions, sister

chromatid reunions and rarely non-sister chromatid reunions, as well as by no reunion at all.

(ii) The frequencies of the breakages differ from those found with induced breakage, so far as can be judged from previous incomplete analyses, only in this respect that they have an internal threshold and a cell uniformity.

(iii) This uniformity depends probably on synchronization of breaks combined with a frequency of breaks which increases as the possibilities of sister reunion improve, perhaps therefore as the sensitive moment of splitting approaches.

(iv) Small acentrics occur with unexpectedly high frequency and without sister reunion. Probably therefore they arise from double breaks with reunion, i.e. as rings, and are favoured in frequency by the ease of non-sister reunion as opposed to restitution, and not by pairs of close breaks arising from single hits.

3. The similarity of natural and induced changes confirms the view that diversity in results of induced breakage is due to diversity in conditions of reunion, especially sister reunion.

4. When allowance is made for this diversity all chromosomes can be shown to behave as though they split uniformly and late in the inter-mitotic resting stage.

5. Analysis of X-ray work on chromosome breakage indicates:

(i) That there is no unconditional healing against non-sister reunion, which like sister reunion can continue indefinitely in certain conditions.

(ii) That the exponential dosage-frequency relation, the intensity and fractionation effects, and the small-fragment excess are due to varying competitions between restitutions and new unions, and not to a mere double character of rearrangement on the one hand or a property of healing on the other.

6. Both resting stage and anaphase types of natural breakage and fusion can be related to the nucleic acid starvation described earlier.

7. Special kinds of natural breakage explain the occurrence of certain specialized evolutionary changes such as diminution and centric fragmentation.

APPENDIX

Terms and symbols of chromosome transformation

1. Bodies are labelled according to the number of their centromeres: *Acentric, monocentric, dicentric*—C_0, C_1, C_2.

2. Where necessary the distinction is made between chromatids and

chromosomes by adding single and double indices: *Acentric chromatid*—C_0'. *Tricentric chromosome*—C_3''.

3. *Loop chromatids* formed by *sister reunion* (**SR**) have the letter l added—C_1'' **l**.

4. *Ring chromosomes* or chromatids without ends are signified by **r**—C_0'' **r** or C_2' **r**.

5. *Telocentric chromosomes* (true or apparent) are denoted by the letter **t**, bridge with fragment by **b**+**f** or C_2+C_0.

6. *Breakage* and *Reunion* are denoted by **B** and **R**. B'' is chromosome breakage. R' is chromatid reunion.

7. *Restitution* is the reunion of two ends which have been parted by breakage.

8. *Sister reunion* (**SR**) is the formation of a continuous chromatid from two sister chromatids as in the formation of an iso-chromosome from a telocentric. This is due to reproductive error of a terminal gene (or centrogene) not to true reunion.

9. *Non-sister reunion* (**NSR**) is the reunion of ends of chromosomes or of chromatids other than sister chromatids.

10. *Healing*, supposititious internal change in broken ends removing their capacity for **SR** on the one hand or for **NSR** or restitution on the other.

Notes (4): All these are descriptions of metaphase chromosomes. A chromosome ring at metaphase becomes a chromatid ring twice as long at anaphase if its chromatids are continuous. Sax (1938, fig. 20) has described as a "dicentric ring chromosome" at anaphase a structure which was a monocentric loop or ring at metaphase (either C_1'' **ll** or C_1'' **rr**). Such description is liable to vitiate the classification based on it. Cf. Text-fig. 21.

(10) Restitution is like healing in that it removes the capacity for **SR** or **NSR**. But, since it is neither supposititious nor internal, to describe it as healing is equivocal and even misleading.

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EXPLANATION OF PLATE 10

Microphotographs of pollen grains, aceto-carmine preparations

Figs. 1–4. Abnormal clone of *Tulipa fragrans* ($n = 12$). Fig. 1. Unbroken chromosomes. Fig. 2, with tetracentric, as in Text-fig. 13 D. Fig. 3, with ring and rod dicentrics, as in Text-fig. 13 C. Fig. 4, as in Text-fig. 12 A. × 1200.

Figs. 5–7. Abnormal fragmenting chromosomes of *Tulipa silvestris* ($n = 24$), photographed at three focuses at anaphase; 5 and 7 show centric fragments at the poles, 6 shows acentrics on the equator. Aceto-carmine. × 1000.
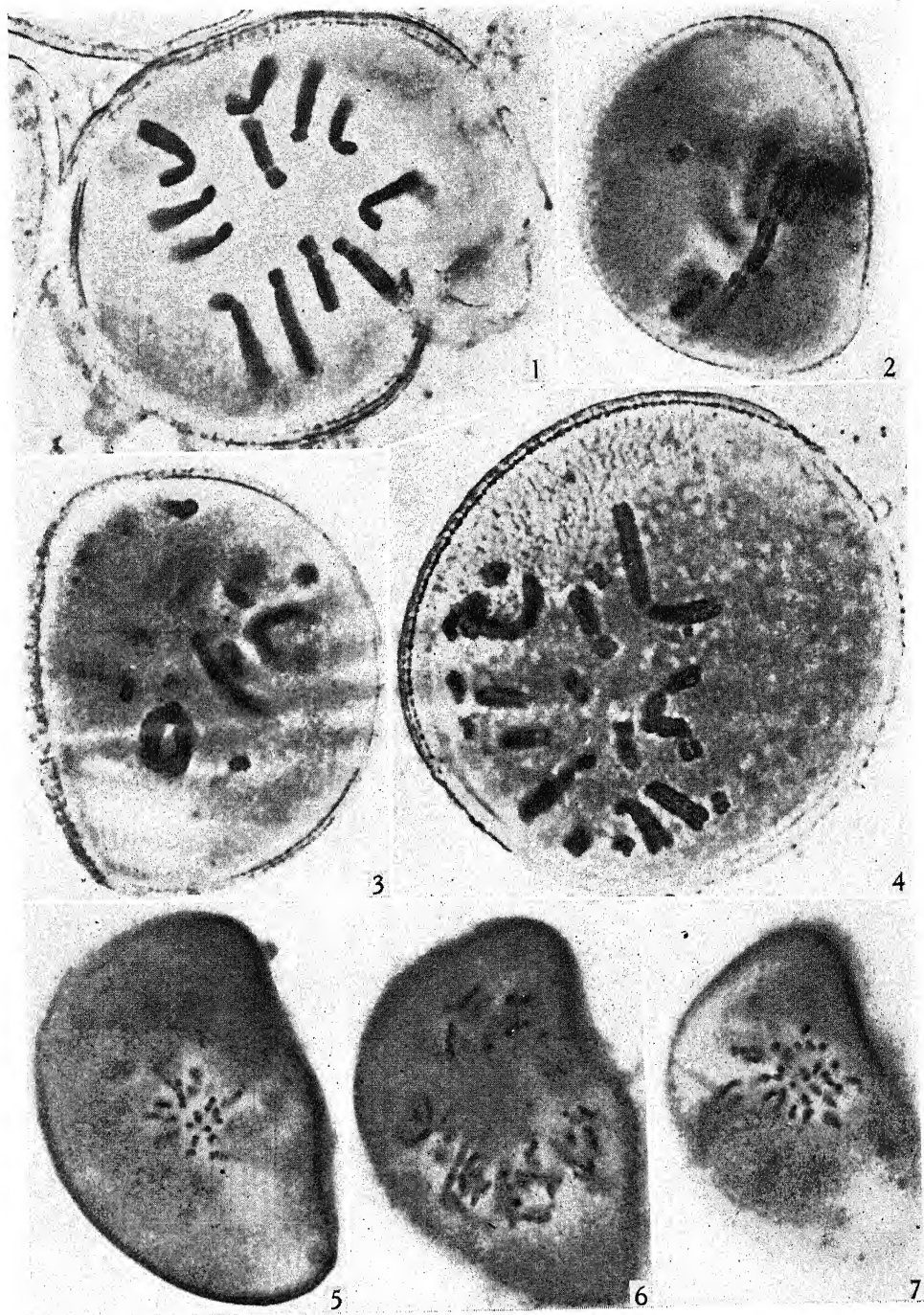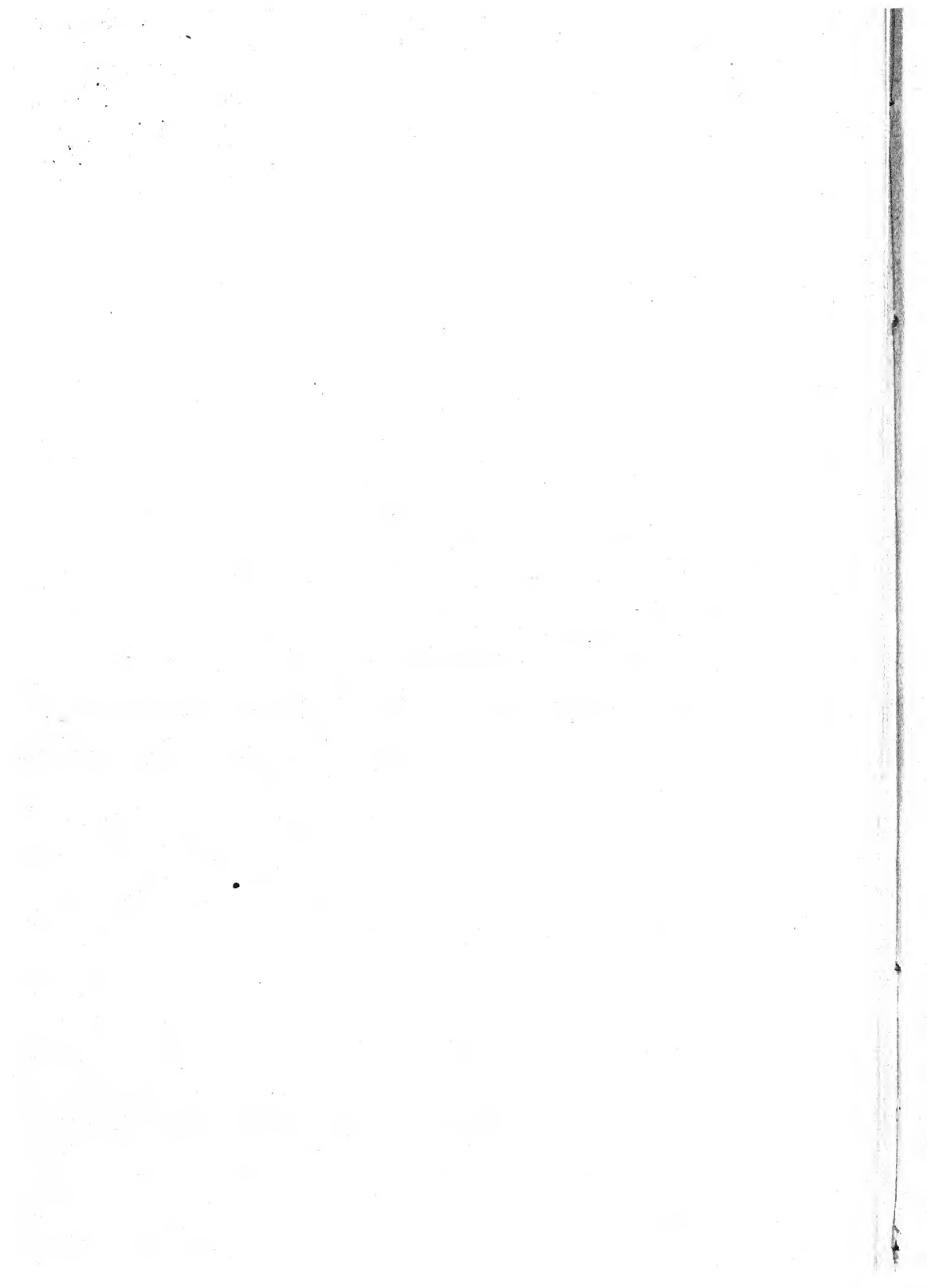
THE GENETICS OF *AQUILEGIA VULGARIS*

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CONTENTS

1. INTRODUCTION

THE common columbine, *Aquilegia vulgaris* L., has been in cultivation for centuries, and like many old garden plants, it shows great variation: a range of colours, doubleness, sepalody, colour pattern, albinism, and variegation. Now some of these variant forms are no doubt derived from the original wild columbine and owe nothing to hybridity; but others may be derived from crossing-out to different species. The plant as it exists in gardens is a complex of uncertain history; and one of the objects of genetic study in such a case is to unravel the complex and discover to what source the components owe their origin. I shall use the name *A. vulgaris* to cover the whole group and shall return later to the question of interspecific relations.

The character differences described in the present paper are due to an independent factor pair **B, b** for blue/pink flower colour, and a linkage group consisting of two pairs and a set of three multiple allelomorphs:

C, c, tall/compact habit.

L, l, blue/lilac flowers.

A, a^w, a, fully coloured/"white face"/fully white flowers.

2. SOURCE OF THE MATERIAL

The history of the stock begins in 1931 when a family 35/29, the progeny from selfing a light blue plant obtained from some cultivated source, was observed to be showing strong coupling of blue colour with tall habit and of lilac colour with compact habit. This family was used

in crosses with a tall and a dwarf white, bought as "nivea grandiflora" and "alba nana compacta" respectively. Later, other stocks were crossed in. 35/29 thus contributed the factors a^w, **b**, **c** and **l**, while the whites contributed **a**, which was at first thought to be an independent factor but later proved to be at the same locus as a^w.

The effect of the factors is as follows:

b and **l** alter the normal blue colour of the flowers. **BL** flowers are blue, **Bl** lilac, while **bL** and **bl** are pink and indistinguishable.

c gives plants about half the height of the normal **C**, with a tighter, bushier habit. Not all dwarfs are **c**. **c** flowers are raised above the horizontal at the time of opening, and turned vertically upwards when they have been open for a day or two. **C** flowers open nodding, and the stems only straighten up after flowering. **C**, **c** can be classified without difficulty on adult plants.

A plants have fully coloured flowers and leaves. **a** plants can be distinguished by the almost complete absence of anthocyanin in their stems and leaves. The flower is usually white or slightly tinged. The intermediate stage, a^w ("white face"), produces fully coloured leaves and sepals, but the petals, when in the normal spurred condition, have a white or creamy-white border about half an inch deep. White face is conspicuous on blue or lilac flowers, much less so on pink.

The colours in 35/29 were the lightest shades found on coloured-stemmed (**A** or a^w) plants. **BL** flowers were a pale sky blue, **Bl** a slaty mauve and **b** flowers a very pale cold pink, flushed with a little mauve on the backs. So pale is the pink that it was not realized until the 1933 families were examined that a^w could be classified, as it can with a little practice, on **b** plants. In 35/29 and the 1931 families, therefore, it is only scored on **B** plants; in later families on **b** as well.

On wholly spurless flowers, where the petals are all sepaloid, a^w is lost as it cannot be distinguished by eye from **A**. On intermediate-spurless flowers the a^w types may show a white central streak on the petals. The spurless (sepaloid) character, however, will not be further discussed here.

To return to **a**, it is possible to get very heavily tinged whites, in which the flower colour may simulate the light blues and pinks of the **A** and a^w series; but the abnormally light foliage is always in evidence. The heavily tinged whites correspond to the darkest, almost black, shades on the **A** stem. In the bulk of my material it is impossible to record **b** or **l** on **a**, but in some strains it may at least be possible to discriminate **B** and **b** (see Discussion, (*a*)).

Crosses between different whites and tinged whites in *vulgaris* have never given anything darker.

As **b** and **l** are lost on **a**, and **l** is lost on **b**, the most useful recessives are the dwarf white-faced purple, $a^w a^w BBccll$, and the corresponding pink extracted from purple, $a^w a^w bbccll$.

3. SINGLE-FACTOR RATIOS

The single-factor ratios of **b**, **c** and **l** are given in Table 1.

Table 1. *Single-factor ratios of* **b**, **c** *and* **l**

Genes	No. of families	Backcross		Deviation from 1 : 1	Hetero-geneity	D.F.	P
		X	**x**				
B, b	17	400	375	0·806		1	0·5–0·3
					12·979	16	0·7–0·5
C, c	18	483	456	0·342		1	0·7–0·5
					21·626	17	0·2–0·1
L, l	17	256	254	0·0078		1	0·95–0·9
					13·686	16	0·7–0·5
		F_2		3 : 1			
B, b	22	764	260	0·083		1	0·8–0·7
					19·656	21	0·7–0·5
C, c	17	491	135	3·909		1	0·05–0·02
					10·349	16	0·9–0·8
L, l	11	280	98	0·173		1	0·7–0·5
					8·096	10	0·7–0·5

The column header χ^2 spans "Deviation from 1 : 1" and "Hetero-geneity".

In the **C, c** selfs one family contributes largely to the total deviation, which calculated on the other sixteen families has $P = 0·2$–$0·1$. The rest of the data give good agreement with expectation.

A is dominant over a^w and **a**, and a^w is dominant over **a**. In every case tested (five families, ninety-five plants in all) white crossed by white face and reciprocally has given nothing but white faces and whole whites in F_1.

If a^w and **a** were not allelomorphic, we should expect that some whites would give a fully coloured F_1 when crossed with white face, and that in the F_2 from a fully coloured plant and a white giving white face on crossing with the latter, some white faces would appear in F_2 or backcross. Actually one white face is recorded in the F_2 family 6/33, but this was probably a stray from the neighbouring family 7/33, where many such occurred. If we accept it at its face value it may be a mutant. The only alternative would be to suppose that the whites were **aa**, the white faces **A ww**, and that the two genes were very closely linked, giving 0·2 % of crossing-over. This is possible, but unlikely. The data on linkage fully bear out the hypothesis of multiple allelomorphism.

The single-factor ratios for **A**, **a^w**, **a** are given in Table 2.

Table 2. *Single-factor ratios of* **A**, **a^w**, **a** *in different types of mating*

Mating*	No. of families	**X**		**x**	Deviation from 1 : 1	Hetero-geneity	D.F.	*P*
Aa × **aa** and reciprocal	4	141		147	0·1450		1	0·8–0·7
						0·3828	3	0·95–0·9
Aa^w × **a^wa^w**	2	89		57	6·918		1	<0·01
						1·195	1	0·3–0·2
a^wa^w × **Aa^w**	6	173		166	0·114		1	0·8–0·7
						7·363	5	0·2–0·1
Aa^w × **aa** and reciprocal	5	115		123	0·268		1	0·7–0·5
						0·550	4	0·98–0·95
		A	**a^w**	**a**	2 : 1 : 1			
Aa × **a^wa** and reciprocal	2	48	21	37	5·774		2	0·1–0·05
						2·580	2	0·5–0·3
		X		**x**	3 : 1			
Aa selfed	15	476		126	5·318		1	0·05–0·02
						11·882	14	0·7–0·5
a^wa selfed	6	189		51	1·800		1	0·2–0·1
						1·826	5	0·9–0·8
Aa^w selfed	8	271		97	0·362		1	0·7–0·5
						10·271	7	0·2–0·1

* Reciprocals throughout are grouped where there is no significant difference between them.

There is on the whole a satisfactory agreement with expectation. In the cross **Aa^w** × **a^wa^w**, where *P* is less than 0·01, most of the deviation is due to a family 14/34, segregating 44 : 33 ($\chi^2_{[1]} = 6\cdot528$).[1] The reciprocal group of six families shows no significant deviation; combination of the two groups would also reduce the deviation to a non-significant figure, but this is scarcely legitimate as the heterogeneity between the two groups of families tested by means of a 2 × 2 table gives $\chi^2_{[1]} = 4\cdot025$. In the **Aa** selfs there is a significant shortage of the recessive class, *P* falling between 0·05 and 0·02; the deviation here is largely due to a group of sister families, 21, 22, 24 and 26/31, unrelated to the main linkage and white lines (see above, Source of the material). If these four families are removed the remaining eleven are a very good fit.

4. LINKAGE OF **c**, **l** AND **a**

The locus of **A**, **a^w**, **a** has proved to be linked with **C**, **c** and **L**, **l**. The backcross data are summarized in Table 3.

[1] The number of degrees of freedom is given in square brackets as a subscript to χ^2.

Table 3. *Linkage of* cl, la *and* ca

Mating	No. of families	XY	Xy	xY	xy	Deviation (χ^2)	Heterogeneity (χ^2)	D.F.	P
$\frac{CL}{cl} \times$ ccll and reciprocal	10	200	12	6	196	345·16		1	<0·01
							1·16	9	<0·99
$\frac{LA}{la^w} \times$ lla^{w}a^w and reciprocal	4	99	10	16	84	117·99		1	<0·01
							1·05	3	0·8–0·7
$\frac{La^w}{1A} \times$ lla^{w}a^w and reciprocal	3	11	44	55	7	55·71		1	<0·01
							0·55	2	0·8–0·7

		LA	1A	La^w	la^w	ba	ba^w	a				
$\frac{La^w}{la}$Bb$\times\frac{1A}{la}$ and reciprocal	2	(17	19)	14	1	(12	5	32)	11·26		1	<0·01
										0·18	1	0·7–0·5

		XY	Xy	xY	xy					
$\frac{CA}{ca} \times$ ccaa and reciprocal	3	110	21	16	110	130·3		1	<0·01
							0·37	2	0·9–0·8
$\frac{CA}{ca^w} \times$ cca^{w}a^w	2	41	5	7	33	44·6		1	<0·01
							1·44	1	0·3–0·2
cca^{w}a$^w \times \frac{Ca^w}{cA}$	2	11	45	67	14	55·24		1	0·01
							0·03	1	0·9–0·8

Table 4 gives additional linkage data from selfed families.

Table 4. *Selfed families involving linkage of* cl, la *and* ca

Constitution of parent	No. of families	Segregation			
CL.cl	3	**CL** 107	**Cl** 2	**cL** 3	**cl** 24
LA.la	1	**LA** 45	**1A** 1	**Ab** (22	**a** 15)
LA.la^w	1	**LA** 23	**1A** 1	**La^w** 3	**la^w** 2
La.1ABb	2	**LA** 36	**1A** 18	**Ab** (14	**a** 23)
La.1ABB[1]	3	79	35	—	(38)
La^w.1A	2	**LA** 36	**1A** 22	**La^w** 27	**la^w** 1
CA.ca	1	**CA** 13	**Ca** 0	**cA** 1	**ca** 3
CA.ca^w	2	**CA** 50	**Ca^w** 4	**cA** 4	**ca^w** 10
Ca.cA	7	**CA** 120	**Ca** 61	**cA** 62	**ca** 2
Ca^w.cA	1	**CA** 18	**Ca^w** 9	**cA** 9	**ca^w** 1

[1] One selfed family plus two families from sister plants crossed inter se.

Table 5 gives the figures obtained from families with all three linked factors segregating.

Table 5. *Linkage of* cla[1]

	CLA.cla^w × cla^w 5 families		CLa^w.clA × cla^w 3 families		Total
Non-recombination	CLA	98	CLa^w	40⎫	272
	cla^w	79	clA	55⎭	
Single recombination in region 1	Cla^w	6	ClA	0⎫	11
	cLA	1	cLa^w	4⎭	
Single recombination in region 2	CLa^w	8	CLA	11⎫	40
	clA	14	cla	7⎭	
Double recombination	ClA	1	Cla^w	0⎫	3
	cLa^w	2	cLA	0⎭	
	Total	209		117	326

The order of the factors is therefore **cla**.

Recombination in region $1 = \frac{14}{326} = 0.0429$.

Recombination in region $2 = \frac{43}{326} = 0.1319$.

Coincidence value $= \dfrac{326 \times 3}{14 \times 43} = 1.62$, which shows no evidence of interference.

The recombination values were calculated from the combined data by the method of maximum likelihood, and a χ^2 appropriate for the detection of heterogeneity between the various bodies of data concerned in each recombination value was calculated in the manner described by Mather (1938, § 18). These values are given below.

$$\text{cl} \quad 4.286 \pm 0.867\,\% \qquad \chi^2_{[1]} = 0.018, \; P = 0.9\text{--}0.8$$
$$\text{la} \quad 13.095 \pm 1.695\,\% \qquad \chi^2_{[6]} = 13.608, \; P = 0.05\text{--}0.02$$
$$\text{ca} \quad 15.235 \pm 1.468\,\% \qquad \chi^2_{[3]} = 2.836, \; P = 0.5\text{--}0.3$$

These values confirm the order **cla** arrived at from the three-point data. In the **la** figures, where the χ^2 is on the margin of significance, most of the heterogeneity is contributed by one family (26/31, $\chi^2 = 10.28$), which contained a single purple plant. I suspect that this plant was a stray and that the family was in fact not heterozygous for **l**. The remaining seventeen families covered by the **la** data give a good fit. If 26/31 was wrongly included the recombination percentage would rise to approximately 13.6.

5. Discussion

(a) *Comparison with the results of Kristofferson*

The only other genetic analysis of *Aquilegia* known to me is that of Kristofferson. He postulates a factor **B** for blue, another factor **R** for

---

[1] These data are included in Tables 3 and 4.

red; **BR** by interaction gives dark blue, **br** is white. This does not appear to square with my results; but I suspect he was working with a deeply pigmented strain where the **a** plants show a heavy tinge (see p. 340). Our classes would then correspond as follows:

Kristofferson		Present scheme	
BR	dark blue	**AB**	blue
Br	light blue	**aB**	white, blue tinge
bR	red	**Ab**	red
br	white	**ab**	white, pink tinge?

My symbol **B**, **b** therefore agrees with Kristofferson's, that is, we both use it to distinguish blue from not-blue. His **R**, however, I have had to discard as it cuts across my **A**, **a** classification. Since in material homozygous for full colour I can self a blue and get a 3 to 1 segregation for blue and red, it is clear that no second factor for red need be invoked. One must postulate the presence, in Kristofferson's material, of an intensifier which will allow **B**, **b** to be separated on **a** plants. Such an intensifier, as stated above, appears to be present in some of my material.

Kristofferson also worked with a character "white margin". It bred as follows:

Dark blue self × white

F_1 18 dark blue self 17 dark blue, white margin

F_2 (3 plants tested) Bred true for white margin
63 self, 20 white margin

He does not state where, if at all, the whites reappeared; but says: "The effect of this factor is seen in the dark blue and red types, and only these have been used in determining the ratio of the segregation." Now in a similar cross with my material, that is, a blue self × white giving blue selfs and white faces, the F_1 blue selfs would segregate blue selfs to white as $3:1$, and the F_1 blues with white face would segregate white face to white as $3:1$. It is possible that when Kristofferson says his white margins breed true, he is ignoring the segregated whites; but it seems impossible to reconcile the breeding behaviour of his tested F_1 blue selfs, which are explicitly stated to have given 63 whole colour to 20 white margin, with my scheme where they would have given no white faces. One must conclude that assuming Kristofferson's account to be correct, his factor **c** is not the same as my **a^w**.

(b) Doubleness of the flower

Various crosses were made to test the relations between doubleness and the linkage group. In family 15/37 there was complete linkage of doubleness with tallness (27 tall double, 10 compact single). On the

other hand in families 7/37 and 8/37, where the doubleness was from the same source as in 15/37, doubleness was independent of white face (segregation 80 : 29 : 22 : 6, $\chi^2_{[1]} = 0.293$, $P = 0.7–0.5$). It seems possible therefore that the parent of the family 15/37 contained an inversion, which would account for such aberrant behaviour.

The inheritance of doubleness, which is somewhat complex, will be reported upon later.

(c) *Interspecific relations*

The typical *Aquilegia vulgaris*, like all the western European columbines, has whole-coloured flowers; the bicolor form is exceptional, and as far as I know is only found in the cultivated plexus. A number of Asiatic and American species, however, normally have bicolor flowers in which the spurred petal is wholly or partly white. It is possible that the white-face form in the common columbine arose independently by mutation; but from what we know of the genetics of garden plants (e.g. *Verbena*, Beale, 1940) it seems more likely that it was introduced from a bicolor species. So far as is known, *A. vulgaris* crosses with every other species of *Aquilegia* (Anderson & Schafer, 1931), and in most cases the hybrids are highly fertile. Subsequent selection for the robust *vulgaris* types might well result, after a few generations, in a plant indistinguishable from the true *vulgaris* except in the flower-pattern character.

If white arose by mutation in *A. vulgaris*, and white face was brought in from another species, the normal linkage behaviour suggests that there have been no great structural changes in the chromosome in question during species formation.

If the white face character has been brought in by a cross, a possible parent would be the Japanese species *A. flabellata*. This was introduced to Europe in the eighties. The original diagnosis says "Sepala coerulea, petala apice flavescentia" (Siebold & Zuccarini, 1846), and this corresponds with the figure in the *Alpine Plants of Japan* (Miyoshi & Makino, 1907), where the sepals are blue and the petals which show are white. Pallid forms are also known, see *Rev. Horticole*, 1887, p. 548, and 1896, p. 109 ("fleurs blanches, légèrement lanées de rose lilacé") and *Curtis's Bot. Mag.* 1911, t. 8354 (flowers white). *A. flabellata* is a reputed parent of *Aquilegia × Helenae* Hort.

A plant received as *Aquilegia × Helenae*, but indistinguishable from *A. flabellata*, having a blue flower with a white edge, was used in some of my early crosses and segregated "white face" of the usual type in F_2. However, white *vulgaris* crossed with a very pale, almost white, *flabellata*

gave blue flowers with white face. This indicates that the white of the *flabellata* used is not at the same locus as that of the *vulgaris*. Either the white face of *flabellata* may be entirely distinct from that of *vulgaris*; or white face and white in *flabellata* may be controlled by two factors, the first of which is allelomorphic with the white of *vulgaris* while the second is independent. It is possible that the very pale *flabellata* used was not a true white, but carried a factor for dilution rather than for albinism. In this case it would not be surprising to find this factor at a different locus from the white of *vulgaris*. Unfortunately the original material has been lost, and the question cannot be settled on the data available.

6. SUMMARY

1. Four sets of factors are described in *Aquilegia vulgaris*:

B, **b**, blue/pink flower colour.

C, **c**, tall/compact habit.

L, **l**, blue/purple flower colour.

A, **a^w**, **a**, whole colour/white face/white flower.

2. **cla** are linked in that order, the recombination percentages being:

cl $4·286 \pm 0·867$.

la $13·095 \pm 1·695$.

ca $15·235 \pm 1·468$.

3. **B**, **b** is independent of the linkage group. Doubleness also is normally independent of it, but is completely linked with **c** in one family, a cytological abnormality being suspected.

Dr Edgar Anderson and Prof. J. B. S. Haldane have taken part in the analysis of the *Aquilegia* experiments. I am obliged to Dr K. Mather for help in the statistical treatment and to Miss M. S. Campbell of the Natural History Museum for a note on the bibliography of *A. flabellata*.

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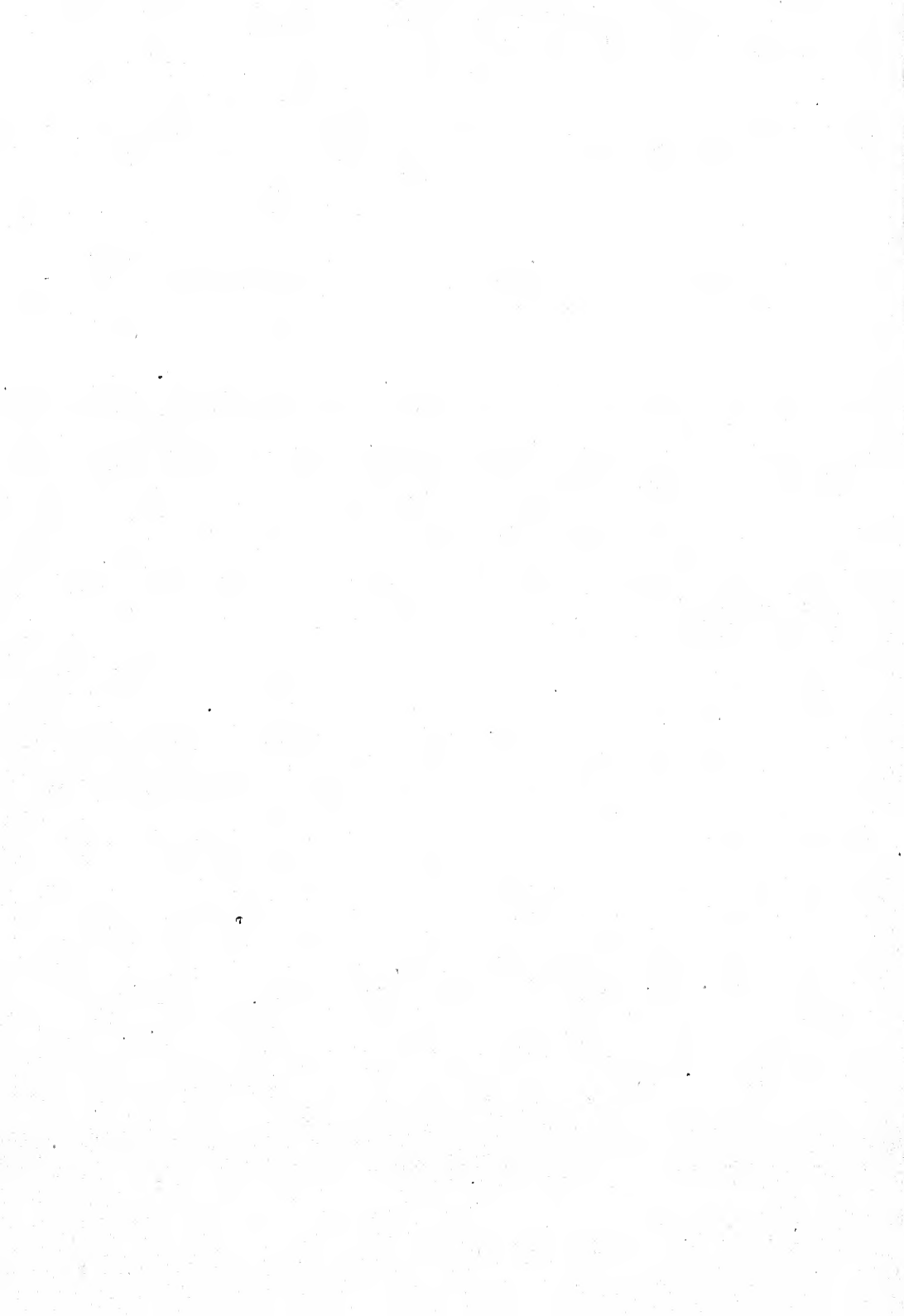
THE SEX-DETERMINING MECHANISM OF THE EARWIG, *FORFICULA AURICULARIA*

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(With Plates 11–13 and Twelve Text-figures)

CONTENTS

PREVIOUS WORK ON SEX DETERMINATION IN DERMAPTERA

THE order Dermaptera has attracted the attention of many cytologists. The chief interest of their work lies in the interpretation of the variability of chromosome number in the male germ-cell line and the complex sex-determining mechanism which exists in some species.

As early as 1885, Carnoy noted that the first and second spermatocytes of *Forficula auricularia* Linn. contained from 10 to 14 "chromosomes". La Valette St George (1887) found 12 "chromosomes" in first and 12–14 in second spermatocytes. Sinéty (1901), on the other hand, gave the male diploid number as 24, haploid 12, without any numerical variability. However, the problem was reopened by Zweiger (1906, 1907) who found 12, 13 or 14 "chromosomes" in first and second spermatocytes. He interpreted the numerical variations as due to varying numbers of sex chromosomes, and hence came to the remarkable conclusion that the sex chromosomes could have no significance in sex determination. Zweiger described occasional lagging chromosomes at the meiotic anaphases, which in his opinion were related in some way to these inconstantly distributed "sex chromosomes".

From 1908 the observations become of importance. They have been summarized in Table 1. The simplest situation is found in the two genera

Table 1. *Summary of work on Dermaptera since 1908*

Species	Author	Mitosis		Meiosis (♂)		Interpretation of sex chromosomes	
		♂	♀	1st	2nd	♂	♀
Labidura bidens	Morgan (1928)	12	12	6^{II}	6	X Y	X X
Labidura riparia	Asana & Makino (1934)	14	14	7^{II}	7	X Y	X X
Labia minor	Morgan (1928)	14	14	7^{II}	7	X Y	X X
Anisolabis maritima	Kornhauser (1921)	25 (24)	26	$11^{II}+1^{III}$	12, 13	$x_1 x_2 Y$	$x_1 x_1 x_2 x_2$
	Morgan (1928)	25	26	$11^{II}+1^{III}$	12, 13	$x_1 x_2 Y$	$x_1 x_1 x_2 x_2$
	Randolph (1908)	24	24	12^{II} ("11, 13, 16, 19")	12	?	?
Anisolabis marginalis	Sugiyama (1933)	25	26	$11^{II}+1^{III}$, $12^{II}+1^{I}$	12, 13	$x_1 x_2 Y$	$x_1 x_2 x_2 x_2$
Anisolabis annulipes	Morgan (1928)	25	26	$11^{II}+1^{III}$, $12^{II}+1^{I}$	12, 13	$x_1 x_3 Y$	$x_1 x_3 x_2 x_2$
Forficula auricularia	Stevens (1910)	24	—	12^{II}	12 (11, 13)	X Y	X X
	Brauns (1912)	—	26	—	—	—	—
	Payne (1914)	25 (24, 26, 27)	25 ?	12^{II}, $11^{II}+2^{I}$, $10^{II}+4^{I}$	12 (11, 13, 14)	XY ?	XX ?
		24 (23)		$12^{II}+1^{I}$, $11^{II}+3^{I}$	12, 13	?	
	Morgan (1928)	25 (26)	24, 25	$11^{II}+1^{III}$, $12^{II}+1^{II}$ ("11")	12, 13	XY ($X = x_1 + x_2$)	XX, $Xx_1 x_2$
Forficula scudderi	Misra (1937)	24	—	12^{II}	12	X Y	X X

I = univalent; II = bivalent; III = trivalent.
Numbers in brackets are counts from exceptional cells: those in inverted commas are of unspecified associations at first metaphase.

Labidura and *Labia*. *Labidura bidens* (Morgan, 1928), $2n = 12$, has five pairs of autosomes and an unequal sex pair in the male. *Labidura riparia* (Asana & Makino, 1934) and *Labia minor* (Morgan, 1928) both have six pairs of autosomes and an unequal sex pair in the male. In all three species the maturation divisions are regular, chromosome numbers are constant and there are no laggards. Since the females have the same diploid numbers as the males, the sex-determining mechanism is clearly of the XY-XX type, the male being heterogametic.

Species of *Anisolabis* form a second group. Three have been examined, and they all possess a complex sex mechanism. The male diploid number is 25, the female 26. At meiotic prophase in the male three chromosomes condense precociously and may all lie in intimate association. *A. maritima* (Kornhauser, 1921; Morgan, 1928) has 12 "chromosomes" at first meiotic metaphase (eleven bivalents and a sex trivalent). The trivalent may be "triangular" or linear in form. The largest member disjoins from the other two at first anaphase and so must be the Y chromosome. At first anaphase the two X chromosomes become detached from each other although passing to the same pole, with the consequence that equal numbers of 12- and 13-type second metaphases are produced.

A. marginalis (Sugiyama, 1933) closely resembles the former species, with the exception that in 1% of the first meiotic metaphases the sex trivalent is replaced by a bivalent and univalent. In *A. annulipes* (Morgan, 1928) the process is carried further, 8% of the first metaphases possessing bivalent and univalent sex chromosomes. When a univalent is present there is nothing to ensure the correct disjunction of the sex chromosomes, but the constancy of the male and female diploid numbers indicates that abnormal complements are eliminated. Morgan notes that in *A. annulipes* disjunction of the trivalent frequently lags behind that of the autosomes. An observation of Kornhauser, the significance of which will be discussed later, is that in certain cysts of the testes of *A. maritima* the spermatogonial divisions show 24 instead of 25 chromosomes.

The results of Randolph's work on *A. maritima* (Randolph, 1908) are at variance with those of the above authors. She found the diploid number of male and female to be 24, and that there were 12 bivalents at first meiotic metaphase in the male, none of which could be recognized with certainty as the sex pair. Occasional cells contained 11, 13, 16 or 19 "chromosomes" at first metaphase. Randolph states that these were abnormal spermatocytes, frequently possessing multipolar spindles, and that second meiotic metaphase was constant in showing 12 chromosomes.

The third group is formed by *Forficula* and here the most extreme complexity exists. The early work has already been summarized. Stevens (1910) has described unequal XY pairs in male material of *F. auricularia* from Heligoland and Eisenach, the diploid number being 24. There were regularly 12 bivalents at first metaphase and this was the normal number for second metaphase. 11- and 13-type second metaphases were found exceptionally, however. Stevens accounts for 11-type second metaphases by assuming that X and Y sometimes pass to the same pole at first anaphase. This would also account for 13-type second metaphases but the latter are sometimes produced in another manner when one of the chromosomes divides equationally at first anaphase. Stevens gives a figure showing such a division. She also figures the remarkable behaviour of a lagging chromosome which sometimes is seen at second anaphase. This chromosome does not divide, but becomes stretched between the daughter telophase nuclei and transected by the newly formed cell boundary.

Payne (1914) worked with *F. auricularia* from Würzburg. His account describes much more variable conditions than does that of Stevens. All but one of his specimens had a spermatogonial number of 24. 12-, 13- and 14-type first metaphases were found, twelve being predominant, and Payne interprets the higher counts as due to unpaired chromosomes, two in 13- and four in 14-type metaphases. Payne hesitates to identify a sex bivalent in all divisions, but he notes one bivalent, one member of which is remarkable in being bilobed at its centric end. He shows that meiotic univalents lag at first anaphase and may divide equationally and he describes and figures a lagging chromosome sometimes found in second anaphases which is clearly of the same type as that noticed by Stevens. One of Payne's specimens had a diploid number of 25, and in this individual first meiotic metaphases showed 12 bivalents and a univalent. In this testis there were abnormal spermatogonial counts, 24-, 26- and 27-type plates being found.

The most complete account of the cytology of male *F. auricularia* is that of Morgan (1928). His material came from Zürich, Switzerland. Many males were examined; 50% had a spermatogonial number of 24, the remainder 25. The meiotic process was very much less variable in 24- than in 25-type males. First metaphase in the former showed 11 autosomal bivalents and an unequal XY pair. Morgan notes, with Payne, that one of these sex chromosomes, the larger, was frequently bilobed. First anaphase was regular, with lagging chromosomes very rare, and the subsequent second metaphase showed constantly 12 chromosomes.

25-type males fell into three classes according to the constitution of first metaphase. In the first class 11 bivalents and a trivalent occurred more frequently than 12 bivalents and a univalent. In the second class there were equal numbers of both, while in the third class 12 bivalents and a univalent were in excess. In most 25-type males there were equal numbers of 12- and 13-type second metaphases, but a few specimens showed a preponderance of 13-type plates, on account of the equational division of a univalent sex chromosome at first anaphase. Morgan notes that there were many lagging chromosomes at first and second anaphases in the 25-type males.

In females of *F. auricularia* mitoses show 24 or 25 chromosomes according to Payne and Morgan, and Brauns has reported 26-types as occurring also. On Morgan's interpretation of the sex-determining mechanism in *Forficula*, the 25-type males are of the constitution $x_1 x_2 Y$, similar in this respect to *Anisolabis*. 24-type differ from 25-type males in that x_1 and x_2 are fused in the former to produce the larger bilobed X member of the XY pair. 24-type females are thus XX, 25-type $x_1 x_2 X$ and 26-type $x_1 x_1 x_2 x_2$.

F. scudderi has been investigated by Misra (1937). This species has $2n = 24$ in the male, and the sex chromosomes form an unequal bivalent at first meiotic metaphase as in the 24-type males of *F. auricularia*. The meiotic process follows the same lines as in the latter, and Misra adopts Morgan's interpretation: that the larger sex chromosome represents a permanent fusion of x_1 and x_2.

MATERIAL AND METHODS

I have worked with specimens of *F. auricularia* Linn. from four different localities: Merton (S.W. London), Wallington (Surrey), Marcham (Berkshire) and Gillingham (Kent). Smears and sections of testes were fixed in La Cour's 2 BD (La Cour, 1937) and stained by Newton's Gentian Violet-Iodine method or by the Feulgen technique. A few sectioned ovaries were also examined.

The testes of *Forficula* are paired organs lying dorsal to the gut. Each consists of two separate follicles which open posteriorly into a common vas deferens. To obtain complete series of meiotic stages, testes of adults were fixed in August. The free end of the follicle at this period contains spermatogonia, with plentiful mitoses. The bulk of the follicle contains first spermatocytes in various stages of meiotic prophase. Posterior to these follows a zone where first and second meiotic divisions

are taking place and then follows a zone of spermatids and finally spermatozoa.

THE TWO TYPES OF MALE

It is clear from the account given by Morgan that there are two cytologically distinguishable types of male *F. auricularia*, $2n = 24$ and 25 respectively. I am able to confirm this (Text-fig. 1). My material shows
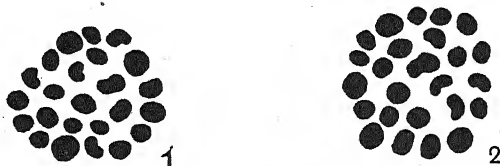
Text-fig. 1. Spermatogonial mitoses in *F. auricularia*. *1*, 24-type; *2*, 25-type. (× 3000.)

the significant fact that in different populations the proportions of the two types vary (Table 2). The proportion of 25-type males in my material falls far short of the 50 % found by Morgan in earwigs from Switzerland.

Table 2. *Proportions of male types in four populations*

Locality	Number of 24-type males	Number of 25-type males	Percentage of 25-type males
Merton (S.W. London)	60	0	0
Marcham (Berkshire)	71	7	9
Gillingham (Kent)	52	13	20
Wallington (Surrey)	15	5	25

I find occasional follicles of spermatogonial mitoses in which the chromosome number does not agree with the "zygotic" diploid number found generally in other follicles of the testis. Thus 25 counts sometimes occur in 24-type males and 24-, 26- and 27-counts in 25-type males. Such irregularity is a characteristic of *Forficula* (as noted by Morgan and Payne), and its significance will be discussed at a later stage.

24- and 25-type males are externally indistinguishable, and undoubtedly belong to the same species of *Forficula*. With Morgan, I can confirm that chromosome number is in no way correlated with the high and low dimorphism of males which exists in this species (Bateson, 1894).

MEIOSIS IN THE 24-TYPE MALE

Prophases of meiosis in *Forficula* fix very well (Text-fig. 2). The leptotene nucleus contains, in addition to the single autosomal chromosome strands, two bodies which stain intensely with Gentian Violet. One of these is irregular in outline, the other of firm outline and shaped like an indian club. The former is Feulgen-positive, and consists of the

sex chromosome pair with a covering of nucleolar material which is Feulgen-negative. The latter is Feulgen-negative and represents the nucleolus. The sex pair come together at the telophase of the last spermatogonial mitosis. They are fully condensed when they associate, and it is highly improbable that their association leads to chiasma formation. In
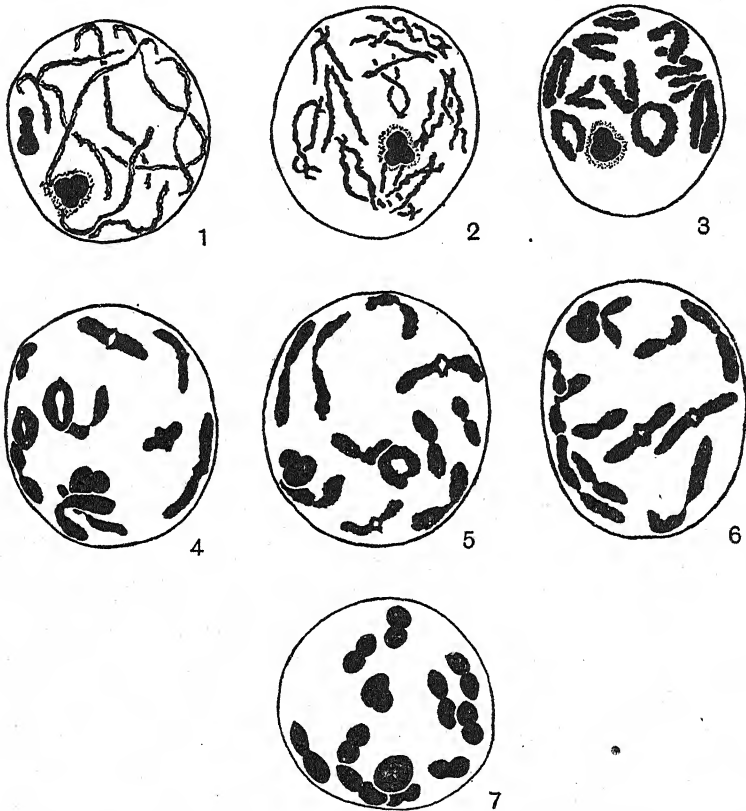

Text-fig. 2. Meiotic prophase in 24-type male. *1*, zygotene (note nucleolus and sex pair); *2*, diplotene (note chiasmata and relational coiling); *3*, later diplotene; *4–6*, early diakineses, 13, 12 and 11 chiasmata respectively; *7*, late diakinesis. (×3000.)

fact it seems likely that their association is conditioned by some non-specific quality which they hold in common, rather than by any precise structural homology since, as will be seen later, associations exist which cannot have arisen by particulate pairing. Autosome pairing at zygotene is unpolarized. Pachytene is a long stage, the diplotene which follows being of much shorter duration. The nucleolus disappears at this stage. Chiasmata can be made out clearly at diplotene: one or two are formed

by each bivalent, but in any one nucleus there are never more than two bivalents which form two chiasmata apiece. A high degree of relational coiling exists between the paired chromosomes at diplotene (Text-fig. 2, *2*). As the nucleus approaches diakinesis, this relational coiling is undone, the chromosomes thicken and the chiasmata terminalize to give rise to dumb-bell or ring-shaped bivalents (Text-fig. 2, *4–6*). Chiasma frequency can be readily estimated at diakinesis. Typical mean values for three different individuals from analyses of 20 nuclei in each are 11·05 (variance = 0·05), 11·3 (variance = 0·43) and 11·7 (variance = 0·64). There is no significant variation from cyst to cyst within one testis.

Further condensation and smoothing of outline of the bivalents occurs during prometaphase. At metaphase the spiralization is so intense that it is not possible to distinguish between one- and two-chiasma bivalents.
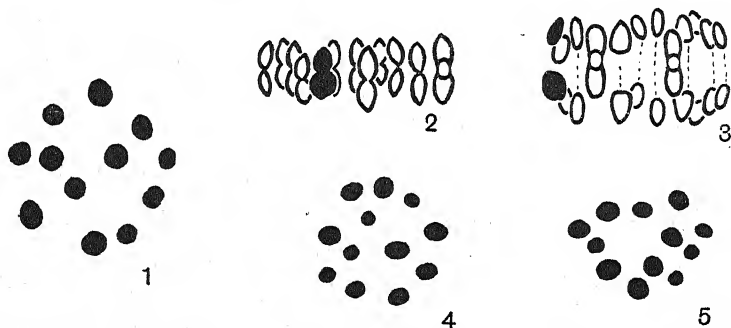
Text-fig. 3. Meiosis in 24-type male. *1, 2*, first metaphase; *3*, early first anaphase; *4, 5*, second metaphase. (× 3000.)

The bivalents become spread out over the metaphase plate, the sex pair always lying at the periphery (Text-fig. 3, *1–3*; Pl. 11, fig. 1). The larger member of the sex pair frequently appears bilobed. The haploid number 12 is almost constant, deviations from it being due, as noted by Payne, to occasional unpaired autosomes. This gives rise to counts of 13 and 14 (Text-fig. 4, *1, 2*). At first meiotic anaphase the sex pair disjoins in time with the autosomes. Sometimes one or two of the latter lag slightly; it is probable that these are bivalents which have formed two chiasmata. In a few anaphases I have seen sticking between one pair of arms of such bivalents (Text-fig. 4, *5*). Univalent autosomes lag at anaphase. They generally divide equationally (Text-fig. 4, *4*).

Second metaphases (Text-fig. 3, *4, 5*) show regularly 12 chromosomes, and the deviations from this number which occur are the results of the equational division of univalents at first anaphase. Second anaphase may show no laggards, daughter univalents passing late and undivided

to one pole or, in some testes not infrequently, a chromosome which becomes stretched between the two telophase groups and subsequently transected by the cell boundary.

The second telophase nuclei subsequently undergo processes leading to the formation of the mature sperm nucleus. The autosomes pass into a diffuse state, the sex chromosomes remaining for some time condensed. Then the nucleus gradually diminishes in volume and increases in stainability, until the highly condensed sperm nucleus is produced.

In a number of 24-type testes I have found cysts with spermatogonial counts of 25 and at first meiotic metaphase an accessary centric sex
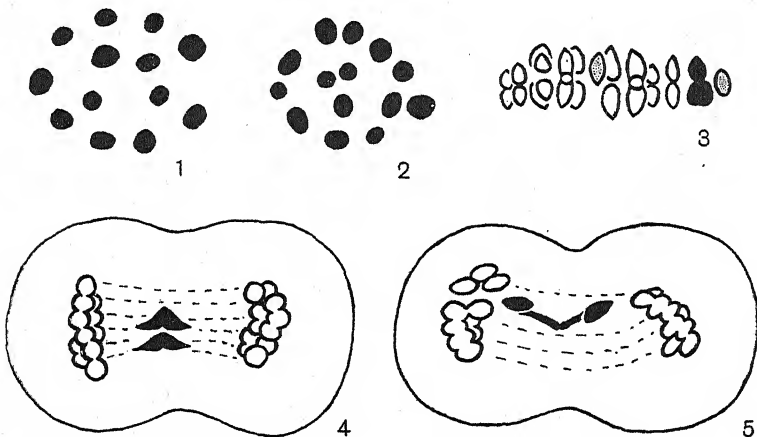

Text-fig. 4. Meiosis in 24-type male. *1–3*, univalents at first metaphase; *1*, 13-type plate; *2*, 14-type plate; *3*, side view, two univalents; *4*, two univalents at first anaphase; *5*, "sticking" of arms of a bivalent at first anaphase. (× 3000.)

fragment (Text-fig. 9, *1, 2*). This fragment must have arisen by an anomalous process at some early spermatogonial division. At first metaphase the fragment is generally seen associated with the sex bivalent. It disjoins with either member of the latter, and at second anaphase divides equationally like any other normal chromosome.

MEIOSIS IN THE 25-TYPE MALE

The behaviour of the autosomes in the 25-type male follows the same lines as in the 24-type male. In the following account the sex chromosomes only will be considered. Any anomalous behaviour of these latter will be superimposed on occasional autosomal abnormalities consequent on univalency at first meiosis, hence making the interpretation of chromosome number in the spermatocytes more complex than in the 24-type.

The leptotene nucleus contains one or two Feulgen-positive bodies and a nucleolus negative to Feulgen. The Feulgen-positive bodies are the sex chromosomes: when there is only one such body, this is the sex trivalent: when there are two, one is bivalent and the other univalent. No further fusion of the sex chromosomes occurs after the telophase of the last spermatogonial division, i.e. a bivalent and univalent condition of the sex chromosomes at leptotene persists until the anaphase of the first meiosis. The form of the trivalent is first clearly recognizable at late diakinesis. It may be "triangular" or linear. That the trivalent may be "triangular" at diakinesis indicates that there is active pairing between
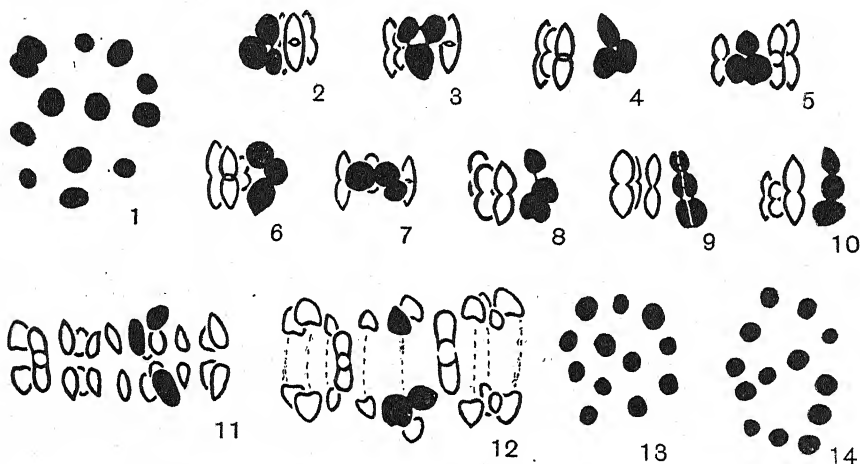
Text-fig. 5. Meiosis in 25-type male. *1*, first metaphase, 12-type plate, trivalent at top left; *2–10*, trivalent at first metaphase in side view; *2–5*, "triangular"; *6–10*, linear; *11, 12*, early first anaphase; *11*, correct disjunction; *12*, mal-disjunction; *13, 14*, second metaphase, 12- and 13-type plates. (× 3000.)

all three component chromosomes: the "triangular" configuration is not merely the result of convergent co-orientation on the first metaphase plate. Lorbeer (1934) has found in one of the liverworts, *Frullania dilatata*, a sex-chromosome trivalent remarkably similar to that of *Forficula* with this same property of pairing between all three members.

At metaphase the sex chromosomes lie at the periphery of the plate (Text-fig. 5, *1–10*; Pl. 11, figs. 2–9). A "triangular" trivalent is usually so orientated that the largest member subtends the other two. This is the disjunction type in *Forficula* as in *Anisolabis* (Text-fig. 5, *11*): it is therefore probable that the largest chromosome is the *Y*. When the three sex chromosomes of the 25-type male are compared with the two of the 24-type, it can be seen that Morgan's "fusion" theory of the

origin of the X in the latter is untenable. This theory supposes that the total volume of the sex trivalent is equal to that of the bivalent in the 24-type male. It is, however, manifestly greater. The size of the individual chromosomes making up the bivalent and the trivalent can be compared by camera lucida drawings after identical treatment of the testes. The largest member of the trivalent is of the same size as the larger chromosome of the bivalent. It frequently appears bilobed, as does the latter. The other two chromosomes of the trivalent are subequal; they are both about the same size as the smaller chromosome of the bivalent. On my view the 24-type male lacks one of the sex chromosomes possessed by the 25-type male. Evidence as to the precise relationship between the two types will be presented later. For the purpose of description, the 25-type will be considered to have the constitution $x_1 x_2 Y$, the 24-type $x_1 Y$ (Y being the larger chromosome which Morgan takes to be the X). 24-type females would thus be $x_1 x_1$, 25-type $x_1 x_1 x_2$ and 26-type $x_1 x_1 x_2 x_2$.

In some 25-type males there is a very high rate of mal-disjunction of the trivalent, due to its incorrect orientation on the first metaphase plate (Text-fig. 5, *12*). This may approach 30%. There is, however, a reason for believing that one form at least of this mal-disjunction gives rise to gametes which can produce viable zygotes at fertilization. Although Morgan found 50% of 25-type males in his material from Switzerland, he found no 26-type females, only 24- and 25-types. 26-type females should have occurred at the same frequency as 24-types in such a population to give a 1:1 ratio of the two male types. This indicates that x_2 may be frequently transmitted by the sperm to the male offspring, i.e. that an $x_2 Y$ gamete is viable. The female material which I have examined consists only of 24-types, but is not sufficiently extensive to be of any significance. Whether the x_2-$x_1 Y$ type of mal-disjunction occurs cannot be certainly stated, though there is no *a priori* reason for believing that it does not. Since no males have been found possessing from the zygote more than one Y chromosome (duplication of the Y when it occurs is not due to an abnormal zygotic complement), the presumption is that the female cannot carry a Y chromosome, and thus $x_1 Y$ gametes, if they occur, fail to produce viable, or at least sexually functional zygotes. In this connexion it may be pointed out that externally normal males without testes are sometimes found. These may perhaps be individuals with anomalous sex chromosome complements. I have also found one specimen, externally female, possessing testes. This proved to be an apparently normal 24-type $x_1 Y$ individual.

The linear trivalent takes on a variety of forms (Text-fig. 5, *6–10*; Pl. 11, figs. 7–9). The three chromosomes may be arranged in a straight line, or bent. This apparently depends on the points of contact between
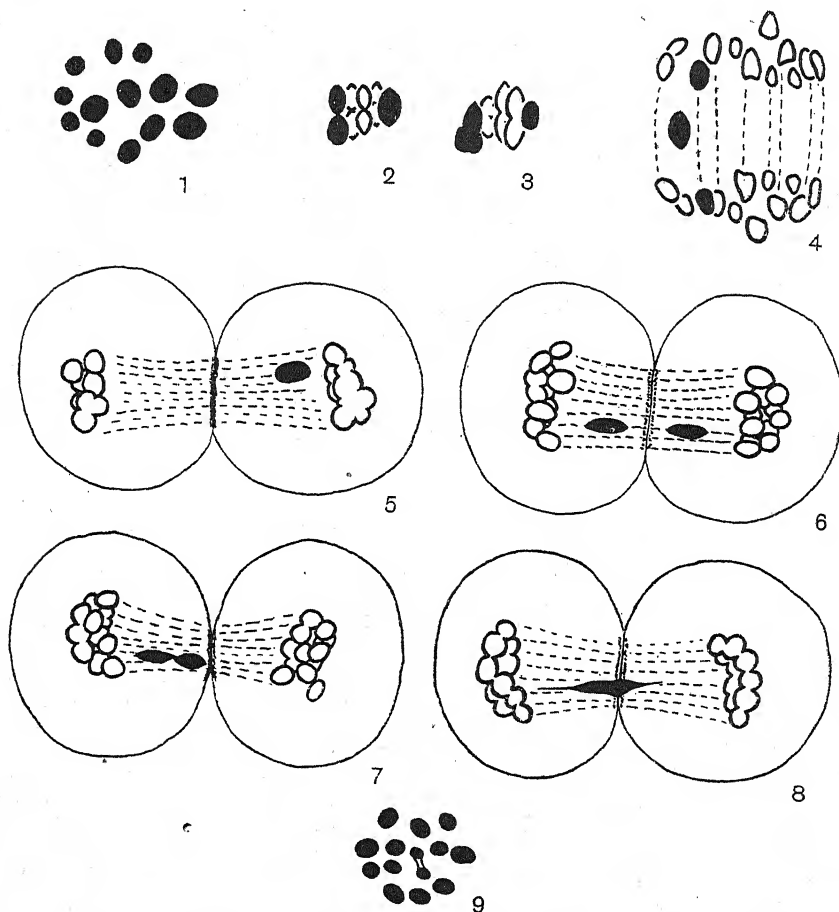
Text-fig. 6. Sex univalent at meiosis in 25-type male. *1*, first metaphase, 13-type plate; *2, 3*, first metaphase in side view; *2*, univalent *Y*; *3*, univalent *x*; *4*, early first anaphase, univalent *Y* at equator; *5–8*, behaviour of univalent *Y* at first anaphase; *5*, passing undivided to one pole; *6*, equational division; *7*, "partial equational division"; *8*, "dicentric stretching"; *9*, 14-type second metaphase derived from anaphase of "partial equational division" type. (× 3000.)

the sex chromosomes, though it is possible that the bent linear trivalent may sometimes result from the breakage of one contact point between the chromosomes of a "triangular" trivalent. The *Y* chromosome generally lies to one end, but it may rarely occupy the middle position.

It is unlikely that this has any significant bearing on the lengths of possible homologous regions, for true pairing does not occur. As with the "triangular" trivalent, the linear trivalent may disjoin correctly or incorrectly.

When the sex chromosomes fail to form a trivalent, a bivalent and univalent being formed instead, it is almost invariable for the Y chromosome to be left as the univalent (Text-fig. 6, *1–4*; Pl. 12, fig. 2). It is thus rare to find a markedly unequal sex bivalent as exists in the 24-type male. However, when such a bivalent is found, it is of the same form as that found in the latter.

The behaviour of the univalent Y at the meiotic anaphases leads me to suppose that it is dicentric. At first anaphase it may do one of four different things:

(1) Pass undivided to one pole (Text-fig. 6, *5*; Pl. 12, fig. 4). This must in any case lead to an illegitimate disjunction type, since x_1 separates from x_2. Further, there is nothing to ensure that Y should pass to the same pole as x_2 rather than x_1.

(2) Divide equationally (Text-fig. 6, *6*; Pl. 12, fig. 3). This results in x_1Y and x_2Y telophase nuclei. A univalent x_1 or x_2 always behaves in this way.

(3) Stretch between the poles of the spindle without dividing (Text-fig. 6, *8*; Pl. 12, fig. 6). This I explain on the assumption that the Y possesses two centromeres which have co-orientated and started to move apart. The boundary formed between the two daughter spermatocytes cuts across the Y chromosome and apparently prevents it from passing to either pole.

(4) Partially complete an equational division (Text-fig. 6, *7*; Pl. 12, fig. 5). Y lingers for a long time at the equator of the spindle. One of its centromeres divides and the two chromatids spring apart. The other undivided centromere, however, prevents the division from being completed. The cell boundary may form and cut across the Y, or the latter may be pulled entire into one or other of the daughter spermatocytes. It may pass to one pole still incompletely divided, or complete the division late.

Counts of second metaphase chromosomes show that 12- and 13-plates predominate (Text-fig. 5, *13, 14*; Pl. 12, figs. 7, 8). These are to be expected to occur in equal numbers as a result of disjunction of the trivalent. Such is sometimes the case, but more generally 13-types outnumber 12-types, and this is clearly the result of equational division of univalent Y chromosomes at first anaphase in a proportion of cells.

11-type second metaphases also occur, but rarely. They may result from the sex chromosomes failing to disjoin at first anaphase. 14-type second metaphases (Text-fig. 6, *9*; Pl. 12, fig. 9), which occur more frequently, are the result of both products of the delayed equational division of *Y* passing to the same pole. In some of these the daughter *Y* univalents remain connected at one point (the undivided centromere) clearly demonstrating their mode of origin.
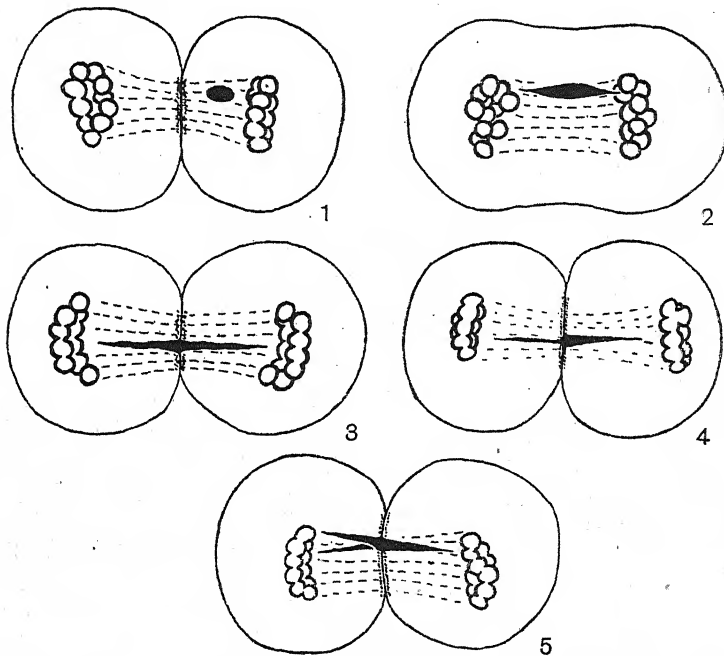
Text-fig. 7. Second anaphase in 25-type male. *1*, daughter univalent passing undivided to one pole; *2–5*, "dicentric stretching" of *Y*. In *5* one of the centromeres has divided equationally. (× 3000.)

Second meiotic anaphases may be quite regular, without lagging chromosomes, or a daughter univalent may pass late and undivided to one pole (Text-fig. 7, *1*; Pl. 13, fig. 1). A more frequent abnormality, however, is for one chromosome to stretch along the spindle between the two poles. This behaviour also occurs (as has been mentioned) in the 24-type male. It is due to "dicentric separation" of the *Y* chromosome. In 24-type males the latter consists of two chromatids. In the 25-type male it may be similarly constituted, or alternatively of only one chromatid, the latter condition being that of a daughter univalent. At early anaphase this chromosome stretches evenly (Text-fig. 7, *2*; Pl. 13, fig. 2).

Later it is transected by the cell boundary formed between the spermatids. The transection may occur about the middle of the chromosome, when the stretching remains even (Text-fig. 7, *3*; Pl. 13, fig. 5). More often, however, the *Y* was nearer one pole than the other when the cell boundary was formed. The result of this is that the smaller portion stretches out into a long thin filament, and the appearance is not unlike the centric misdivision of an almost telocentric chromosome (Text-fig. 7, *4*; Pl. 13, figs. 3, 4). Sometimes, when the stretching *Y* consists of two chromatids, one limb may split owing to the delayed equational division of its centromere. The cell boundary prevents the split from spreading into the other limb (Text-fig. 7, *5*). Rarely, second anaphase may show two such stretching chromosomes. These are presumably the undisjoined daughter univalents of a *Y* chromosome. It is unlikely that a dicentrically stretched *Y* chromosome is ever included in the spermatid nuclei. The cell boundary prevents it from reaching the poles of the spindle, and fragments do not appear to break loose. Presumably this chromosome is shed when the cytoplasm of the sperm is sloughed at the formation of the mature sperm. Were viable zygotes to be produced from gametes carrying fragments of a sex chromosome, they would be noticed in testis fixations. But sex fragments when they appear in the spermatocytes are not general to the whole testis, and they have a different origin.

The hypothesis that *Y* is dicentric cannot, unfortunately, be tested by examination of the mitotic chromosomes, since these are as much condensed as the meiotic chromosomes even in embryo divisions. The bilobed appearance of the *Y* at meiosis, however, provides some evidence as to the validity of the hypothesis.

Table 3 *a, b* shows the frequency of the different types of behaviour of the sex chromosomes in eight 25-type males taken at random. It is important to realize, however, that conditions obtaining in one cyst may differ markedly from those in other cysts of the same testis. The significance of this is obscure. It is thus not possible, for example, to compare accurately the relative frequency of equational divisions of univalent *Y* chromosomes at first anaphase with the excess of 13-type second metaphases. Specimen no. 5 showed in one cyst of second metaphases equal numbers of 12- and 13-counts, in another a large excess of 13-counts. However approximate agreement exists. The relative frequency of linear trivalents to those of the "triangular" type bears an approximate proportionality to sex univalent frequency. In this connexion it may be noted that *Anisolabis* spp. with low univalent frequency also show comparatively few linear trivalents. This indicates that the linear trivalent

Table 3

(a) Sex-chromosome behaviour at first meiosis in 25-type males of Forficula auricularia

Eight individuals arranged in order of decreasing sex-univalent frequency
G = Gillingham; M = Marcham

| | First metaphase | | | | | | First anaphase | | | | | |
| | Polar views | | | Side views | | | | | | | | |
Specimen	11 bivalents + trivalent	12 bivalents + univalent	Frequency of sex univalent %	"Triangular" trivalent	Linear trivalent	Frequency of linear trivalent %	No laggards	Univalent to one pole (Y)	Equational division of univalent (x or Y)	"Partial equational division" of univalent (Y)	"Dicentric stretching" (Y)	Frequency of equational division %
1 (G)	22	13	37	43	12	22	22	7	7	2	2	18
2 (M)	44	24	35	60	20	25	40	23	6	1	0	9
3 (M)	54	22	29	58	15	21	16	11	6	1	0	18
4 (G)	29	7	19	55	8	15	41	8	2	0	3	4
5 (G)	26	5	16	42	10	19	20	6	2	0	1	7
6 (M)	35	2	5	53	3	5	40	3	0	1	0	0
7 (M)	47	0	0	40	3	7	44	8	1	0	0	2
8 (G)	29	0	0	27	2	7	35	2	4	0	5	9

(b) Sex-chromosome behaviour at second meiosis in 25-type males of Forficula auricularia

Eight individuals arranged in the order of (a) above

| | Second metaphase | | | | | Second anaphase | | | | |
Specimen	11-type	12-type	13-type	14-type	Frequency of excess 13-types %	No laggards	Daughter univalent to one pole (x or Y)	"Dicentric stretching" (Y)	Uncertain types	Frequency of "dicentric stretching" %
1 (G)	0	13	22	2	26	57	0	20	5	26
2 (M)	2	65	105	0	42	60	16	0	0	0
3 (M)	1	24	40	1	25	26	4	0	1	0
4 (G)	0	27	35	1	13	49	10	0	1	0
5 (G) Cyst 1	0	10	23	0	39	44	0	27	0	38
5 (G) Cyst 2	0	29	28	0	0	47	0	1	0	2
6 (M)	0	39	42	0	4	73	2	0	0	0
7 (M)	0	29	37	0	12	52	2	2	3	4
8 (G)	0	20	25	0	11	42	0	2	0	5

and the sex univalent are both manifestations of reduced "pairing power". A further point of interest is that dicentric stretching of the Y at first and second anaphases does not occur in all testes, indicating that the Y chromosome is not identical in all males of *Forficula*.

Loss, duplication and fragmentation of the Y chromosome

The instability of mitotic chromosome number in *Forficula* is explicable, on the supposition that Y is dicentric, in the following way. Intercentric relational coiling would account for abnormal mitotic behaviour leading to loss, duplication or fragmentation of the Y chromosome.
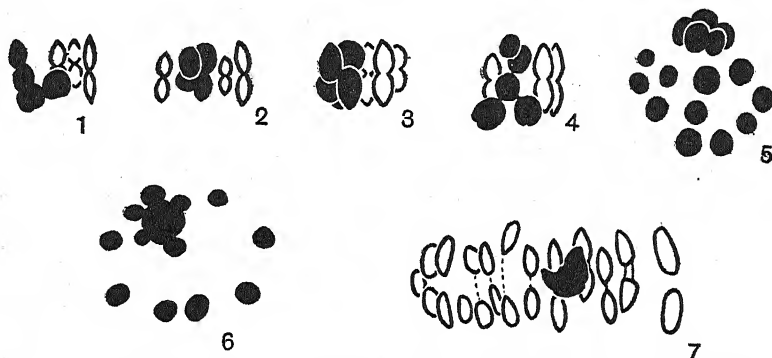
Text-fig. 8. Reduplicated Y at first metaphase in 25-type male. *1, 2*, sex quadrivalent; *3–5*, sex quinquevalent; *6, 7*, anomalous reduplication. (× 3000.)

In 25-type males I have noticed three testes where in one or two cysts the Y chromosome was missing. In these cysts at first meiotic metaphase x_1 and x_2 form an almost equal sex bivalent. In two of these testes nearby cysts contain cells of the complementary type with two Y chromosomes, which associate with x_1 and x_2 to form a sex quadrivalent at first metaphase (Text-fig. 7, *1, 2*). It would thus appear that at an early spermatogonial division the daughter Y chromatids failed to separate and passed together to the same pole.

I have also seen two cysts where there were five sex chromosomes in association in all the cells at first metaphase (Text-fig. 8, *3–5*; Pl. 13, fig. 8). Fixation was poor in both cases and it is not possible to state whether fragmentation of one of the Y chromosomes, or alternatively successive reduplication due to failure of anaphase separation, was responsible. The latter supposition is more plausible.

The sex chromosomes in such quadrivalents and quinquevalents associate in indiscriminate conformations. Centric repulsions are not

adjusted, consequently at first anaphase they lag behind the autosomes and disjoin in a highly irregular manner (Pl. 13, fig. 9).

In the third testis where the *Y* chromosome was missing in one cyst, a nearby cyst contained spermatocytes at first metaphase with a structure which I was unable to interpret. The chromosomes were arranged in a hollow spindle, 12 bivalents and a large body of irregular shape to which some of the bivalents were attached (Text-fig. 8, *6, 7*; Pl. 13, fig. 10). At anaphase disjunction was very irregular due to the lagging of this body and its attached bivalents.

In 24-type males (as has been mentioned) and in 25-type males more frequently, cysts of spermatocytes containing a sex fragment as well as the normal complement may be found side by side with normal cysts.
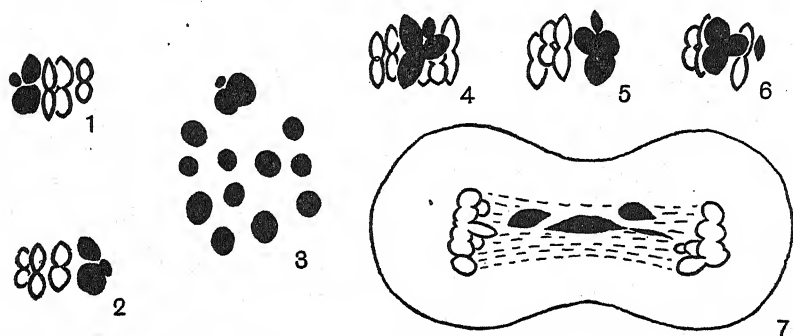
Text-fig. 9. Centric *Y* fragment in 24- and 25-type males. *1, 2,* associated fragment at first metaphase in 24-type male; *3–6,* associated and non-associated fragment in the 25-type male; *7,* associated fragment at first anaphase in 25-type male. (× 3000.)

I interpret these fragments as being parts of the *Y* chromosome broken at a spermatogonial mitosis owing to intercentric coiling. They are generally seen associated with the sex chromosomes at first metaphase, though occasionally they may lie separate (Text-fig. 9, *1–6*; Pl. 13, fig. 6). An associated fragment may or may not interfere with the disjunction of the sex chromosomes (Text-fig. 9, *7*; Pl. 13, fig. 7). A non-associated fragment divides equationally at first anaphase and passes to one pole at second anaphase.

It is important to realize that these are not "zygotic" abnormalities, i.e. the result of fertilizations where the gametes possessed abnormal complements. Only one case of the latter has been found. In this $x_1 x_2 Y$ male there were two autosomal fragments in all the cells of both testes (Text-fig. 10; Pl. 13, figs. 11, 12). These fragments remained unpaired at first metaphase. They divided equationally at first anaphase without lagging, so that all the second metaphases also showed both fragments.

At second anaphase they lagged behind the other chromosomes and passed undivided to one or other pole. They may have originated from autosomal misdivisions at the maturation divisions of the parental sperm or egg.

A striking feature of the testes of *Forficula* is the large number of cysts of necrotic spermatogonia. It is possible that these spermatogonia possess abnormal complements and are unable to compete successfully with their balanced neighbours.
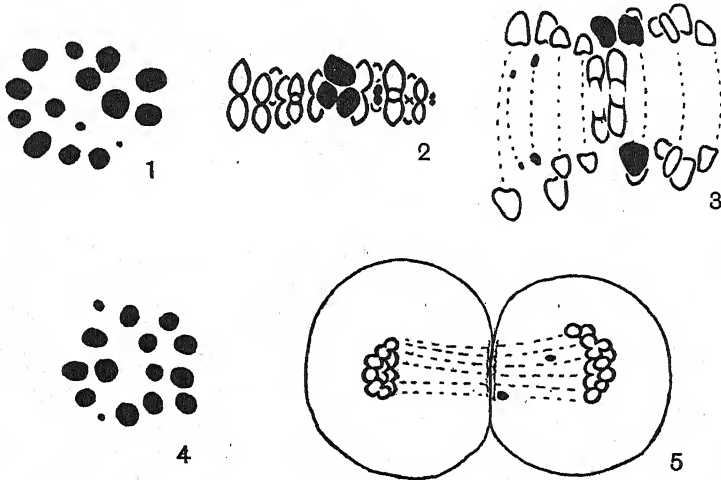
Text-fig. 10. Two autosomal fragments in 25-type male. *1, 2,* first metaphase; *3,* first anaphase; *4,* second metaphase; *5,* second anaphase. (× 3000.)

POLYPLOID CELLS

The testes of *Forficula* contain polyploid cells of various kinds. These may be classified as follows:

(1) *True tetraploid first spermatocytes.* These cells are the products of division of a "doubled-up" spermatogonium. The "doubling-up" may occur early or late in the spermatogonial lineage, for various numbers of such tetraploid spermatocytes may be found together. These tetraploid cells are characterized by the presence of multivalent chromosome associations at first metaphase: chiasma formation will be described in a later paper. They give rise to clumps of diploid second spermatocytes.

(2) *Syndiploid first spermatocytes* (Makino, 1939). These cells are produced when two diploid diakinetic nuclei break down for metaphase orientation, all the chromosomes passing on to a common spindle. They occur singly in an otherwise diploid cyst, and possess no multivalent associations. They give rise to pairs of diploid second spermatocytes.

(3) *Restitution diploid second spermatocytes.* This type of polyploid cell occurs extremely commonly in testes of *Forficula* (Text-fig. 11, *1*; Pl. 2, fig. 10). It is produced when first meiotic anaphase has failed to separate the telophase nuclei completely, and no cell boundary has been formed. This may occur when the first metaphase complement was normal, when there were univalents or when some abnormal first meiotic association was present which prevented disjunction (e.g. a duplicate *Y* chromosome). Restitution diploid second spermatocytes occur singly or in groups. Chromosome number may be slightly in excess of the diploid number if, at the previous anaphase, certain univalent chromosomes divided equationally. Many of these cells possess tri- or tetra-polar spindles: both centrosomes of the preceding division have persisted,
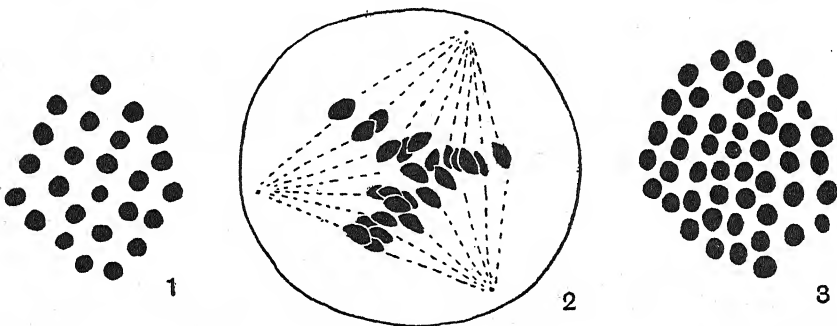
Text-fig. 11. Restitution second spermatocytes. *1*, diploid second metaphase; *2*, diploid second metaphase with tripolar spindle; *3*, tetraploid second metaphase. (× 3000.)

with their capacities to organize spindles, and one or both have divided (Text-fig. 11, *2*; Pl. 12, fig. 11). When a normal dipolar spindle is formed in these second spermatocytes, it is too small to accommodate all the chromosomes present in a flat plate: the plate is almost always curved. This is in contrast to the flat plates of tetraploid first spermatocytes. Restitution second spermatocytes may also occur after the division of a tetraploid first spermatocyte, and thus tetraploid second spermatocytes are occasionally produced (Text-fig. 11, *3*).

THE SEX MECHANISMS OF *ANISOLABIS* AND *FORFICULA*

The $x_1 x_2 Y$ sex mechanism of *Anisolabis* appears to have been the original sex mechanism of *Forficula*. That the number of *x* chromosomes may be one or two but not more, and that *Anisolabis* spp. generally possess two, indicates that the two *x* chromosomes are not identical. We are not dealing here with a reduplication of like chromosomes such

as occurs in *Cimex* (Darlington, 1939b) but rather with a multiple x mechanism similar to that which exists in some Mantids (Oguma, 1921; White, 1940). *Forficula* differs from these, where both x chromosomes are essential, since in the zygote only one x is necessary to determine a viable and fertile male. The other x is superfluous in sex-determination, but is not necessarily inert.

The sex trivalents of *Paratylotropidia* (King & Beams, 1938) and of certain Mantids (White, 1940) are thought to have been the results of interchanges between the sex chromosomes and autosomes. A similar interpretation is possible for the trivalent of *Forficula*. The extreme degree of positive heteropycnosis at meiosis in the male makes an analysis of pairing impossible. In fact, as has already been seen, the "pairing" which exists is not true particulate pairing. Nevertheless, the fact that any one of the three can pair with either or both of the others indicates that there was probably an original partial homology between all three. The dicentric Y provides us with a clue as to how the mechanism could have arisen. Let us suppose that the primitive sex mechanism in the Dermaptera consisted of simple X and Y chromosomes. *Labidura* and *Labia* may have retained this mechanism, or alternatively their simplicity may be secondary. Both X and Y possessed two arms, one of the pairs of arms being homologous, the other differential. One arm of an autosome became translocated on to the differential arm of X to give x_1. The remainder of the autosome, with its centromere, became translocated on to the differential arm of Y. The homologue of this autosome, with pairing regions homologous to parts of both x_1 and Y, would then form the third member of the trivalent, x_2 (Text-fig. 12). The intercentric region of Y, being differential, would escape breakage due to chiasma formation between Y and the x chromosomes and it would merely have to withstand the possibility of discordant behaviour during mitoses in the male. Reduction of intercentric coiling would be necessary to achieve this, but both *Forficula* and *Anisolabis* show that Y is not always successful in passing through its mitotic divisions without non-disjunction or fragmentation. In this connexion it is interesting to note that Tanaka (1940) has shown that two types of *Scirpus lacustris* possess dicentric chromosomes. These dicentrics behave irregularly at meiotic anaphase: they are associated by chiasmata and frequently break. However, reproduction is doubtless largely vegetative and the permanence of the dicentric within a clone indicates that its behaviour at mitosis is regular.

The sex trivalent as we now see it is probably very different from its

ancestral condition. Strict pairing homology has been lost, and to compensate for this a new mechanism of pairing has arisen involving precocious condensation of the whole of the sex chromosomes, original pairing regions and differential regions alike.

In a trivalent where all three chromosomes are "paired" there must be a special centric mechanism to ensure that orientation and disjunction at meiosis are regular. The dicentric Y is perhaps adapted to perform this function. There are four centromeres in the trivalent which
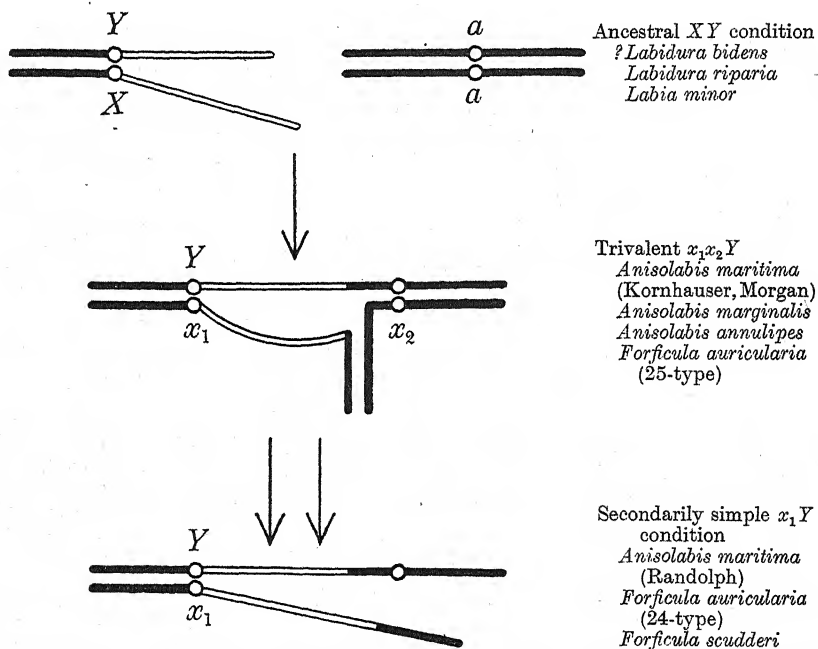
Text-fig. 12. Diagram illustrating the possible evolution of sex chromosomes in the Dermaptera. Primitive pairing regions are represented by full lines. a = autosome.

can effectively repel one another in pairs. Two of these, since they lie on the same chromosome, will behave as a unit at meiosis if their nearness to one another bears a certain relation to the proximity of their respective mates. The irregular disjunction of the trivalent in *Forficula auricularia* is probably secondary; strict accompaniment of x_1 by x_2 is no longer essential and misdivision of one of the Y centromeres may have unbalanced the delicately adjusted mechanism. We can assume that the centromeres of the Y chromosome are not invariably of the same strength (Darlington, 1939a) since the frequency with which dicentric separation of the Y occurs varies considerably from one testis to another.

When the sex chromosomes fail to pair completely and one is left as a univalent (generally the Y) the latter chromosome is placed in a new mechanical situation to which its dicentric nature is not directly adapted. Hence the Y may behave in an anomalous manner at first or second anaphase. Incomplete pairing permits the separation of x_1 from x_2 and this was no doubt the way in which types lacking x_2 came into being. Thus the 24-type male *Forficula auricularia* has reverted to a simple sex mechanism (Text-fig. 12, *3*). On my interpretation in *F. scudderi* the reversion has been completed. The same evolutionary step would explain the account of meiosis in *Anisolabis maritima* given by Randolph, which so flatly contradicts that of Kornhauser and of Morgan. She was presumably dealing with a population where x_2 had been lost.

If in *Forficula* types can be produced which lack the second x chromosome, and these types perform the meiotic process in a regular manner, it is important to understand the selective advantage possessed by the 25-type male whose meiosis is remarkable for its extreme eccentricity. If the 25-type male possessed no such advantage, it would presumably have become extinct, owing to inferior sperm production. Perhaps, as suggested by Darlington to explain the presence of the supernumerary x chromosomes of *Cimex*, x_2 is not inert, and the 25-type male is more viable than the 24-type. The situation in *Cimex* is to some extent analogous, for here selection seems to favour a high supernumerary x complement, yet when there are too many of these the meiotic process cannot deal with them efficiently. On the other hand there is another explanation which appears to me more plausible. Brindley (1912) has made an exhaustive study of the sex ratio in wild populations of *Forficula auricularia*. There is almost without exception a male deficiency, which varies in magnitude between different populations. When his counts are summated, the male : female ratio is approximately 45 : 55 (total counted $= 31820$), while in one locality, Round Island, Scilly Isles, the males form only 16 % of the total population (total counted $= 3655$). A low proportion of males may be of considerable selective advantage to an organism such as *Forficula*; populations are generally dense and the animals are active, so that fertilization is easily accomplished and one male may serve many females. This selective advantage would be roughly proportional to the density of the population. In less dense populations the selective advantage would be less and vice versa. The Y chromosome is lost at a high rate in these 25-type males, hence there is a deficiency of male-determining sperm. On this view the 25-type male would be somewhat analogous to the male of *Drosophila* spp. carrying the sex-

ratio gene (Sturtevant & Dobzhansky, 1936). It will be of great interest to find out the proportions of 24- and 25-type males in populations with extreme male deficiency.

The 25-type male of *Forficula* must produce a high proportion of unbalanced gametes. An estimate of this proportion cannot unfortunately be made, since x_1 cannot be distinguished from x_2 cytologically. White has found a similar situation in certain Mantids (White, 1940) where incorrect disjunction of a sex trivalent is again the cause. Gametic selection in animals has never been demonstrated; zygotic selection, though far more wasteful for the species, has always been proved or presumed when the parents produce unbalanced gametes. On this supposition a mechanically inefficient and wasteful sex-determining system such as exists in the 25-type male of *Forficula auricularia* must have strong compensatory advantages of which sex-ratio adjustment is probably the most important. The dicentric Y is, I suggest, a primitive endowment which has survived as a "long-range" adaptation to the species.

SUMMARY

1. *Forficula auricularia* has eleven pairs of autosomes. There are two types of male: $x_1 Y$ and $x_1 x_2 Y$, and three types of female: $x_1 x_1$, $x_1 x_1 x_2$ and $x_1 x_1 x_2 x_2$.

2. The sex chromosomes in the male pair at the telophase of the final spermatogonial mitosis. They associate by mass, not particulate, pairing. In the $x_1 x_2 Y$ male there may be pairing between all three to give a "triangular" or linear trivalent, or pairing may be incomplete, when a bivalent and univalent result.

3. The Y chromosome is dicentric, judged by its shape at first metaphase and behaviour at first and second anaphases. It may fragment and non-disjoin at the spermatogonial mitoses. The dicentric Y is adapted to orientate the "triangular" trivalent.

4. Owing to incomplete pairing of the sex chromosomes in the $x_1 x_2 Y$ male meiosis is irregular. The Y chromosome may be lost with high frequency. Different relative numbers of $x_1 Y$ and $x_1 x_2 Y$ males may thus adaptively modify the male proportion to suit the population density.

5. Of the two types within this species, the $x_1 x_2 Y$ male represents the primitive condition. The nearly related genus *Anisolabis*, with possibly one exception, possesses only $x_1 x_2 Y$ males. *Anisolabis* females are of the constitution $x_1 x_1 x_2 x_2$.

6. In *Forficula auricularia* x_2 has become superfluous as regards sex determination. When the Y fails to pair with x_1 and x_2 these form a bivalent and disjoin from one another at first anaphase. This accounts for the origin of males lacking x_2. In *Forficula scudderi* x_2 has been lost entirely.

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EXPLANATION OF PLATES 11–13

Microphotographs. All × *ca.* 3500.

PLATE 11

Fig. 1. First metaphase of meiosis in 24-type male: side view showing sex bivalent.

Figs. 2–10. First metaphase of meiosis in 25-type male. Figs. 2–6. "Triangular" trivalent in side view. Figs. 7–9. Linear trivalent in side view. Fig. 10. Polar view of 12-type plate with trivalent at left.

PLATE 12

Figs. 1, 2. First metaphase in 25-type male. Fig. 1. Polar view of 13-type plate. Fig. 2. Side view showing univalent *Y*.

Figs. 3–6. Behaviour of univalent *Y* at first anaphase in 25-type male. Fig. 3. Equational division. Fig. 4. Passing undivided to one pole. Fig. 5. "Partial equational division." Fig. 6. "Dicentric stretching".

Figs. 7–9. Second metaphase in 25-type male. Fig. 7. 12-type. Fig. 8. 13-type. Fig. 9. 14-type.

Figs. 10, 11. Restitution second metaphases in 25-type male. Fig. 10. Polar view. Fig. 11. Side view showing tripolar spindle.

PLATE 13

Figs. 1–5. Behaviour of daughter univalent *Y* at second anaphase in 25-type male. Fig. 1. Passing undivided to one pole. Fig. 2. "Dicentric stretching", early. Figs. 3–5. "Dicentric stretching", later. Cell boundary has formed.

Figs. 6, 7. Centric *Y* fragment in 25-type male. Fig. 6. Side view of first metaphase. Fig. 7. First anaphase.

Figs. 8, 9. Reduplicated *Y* in 25-type male. Fig. 8. Side view of quinquevalent at first metaphase. Fig. 9. First anaphase. Irregular disjunction.

Fig. 10. Anomalous reduplication of *Y* in 25-type male. "Sticking" of bivalents and hollow spindle.

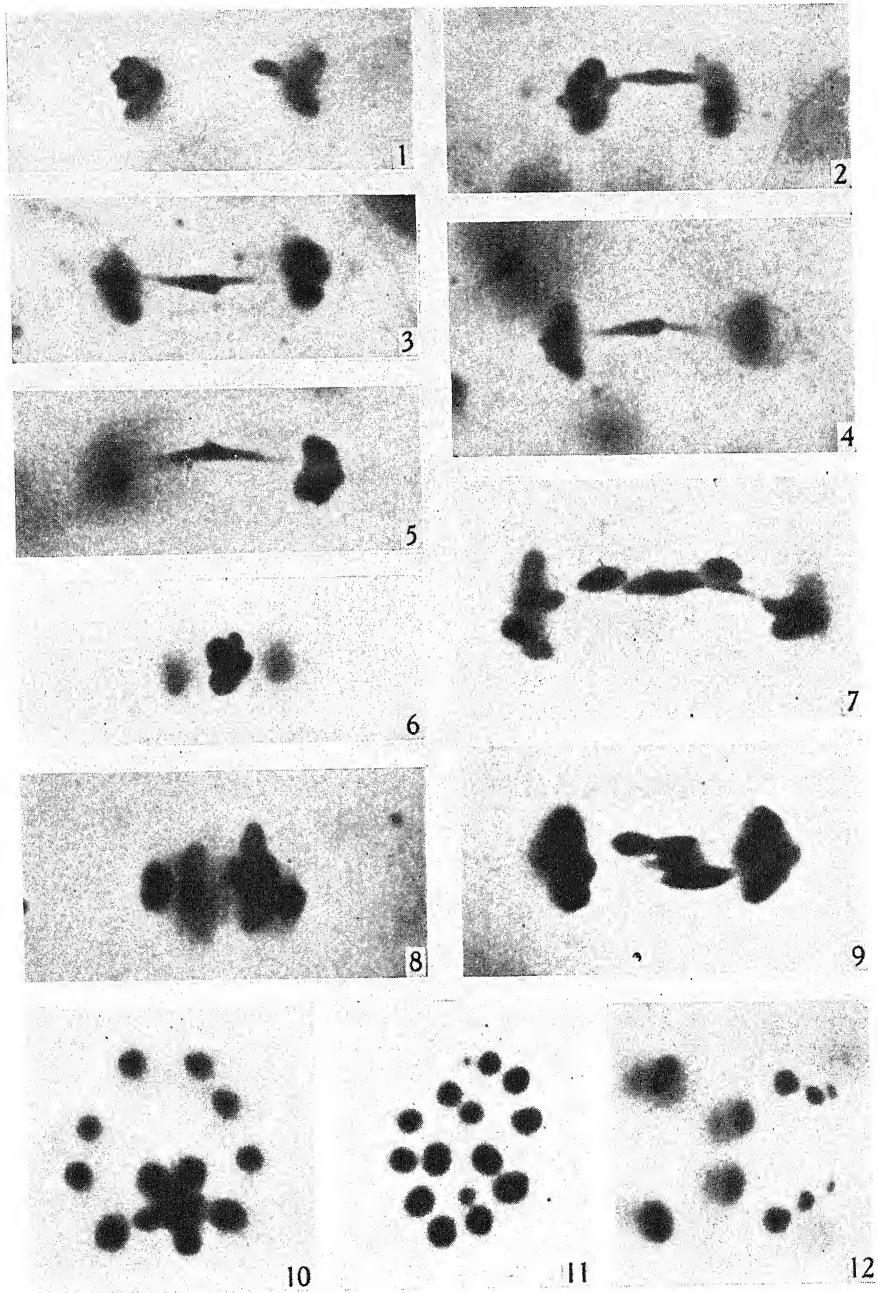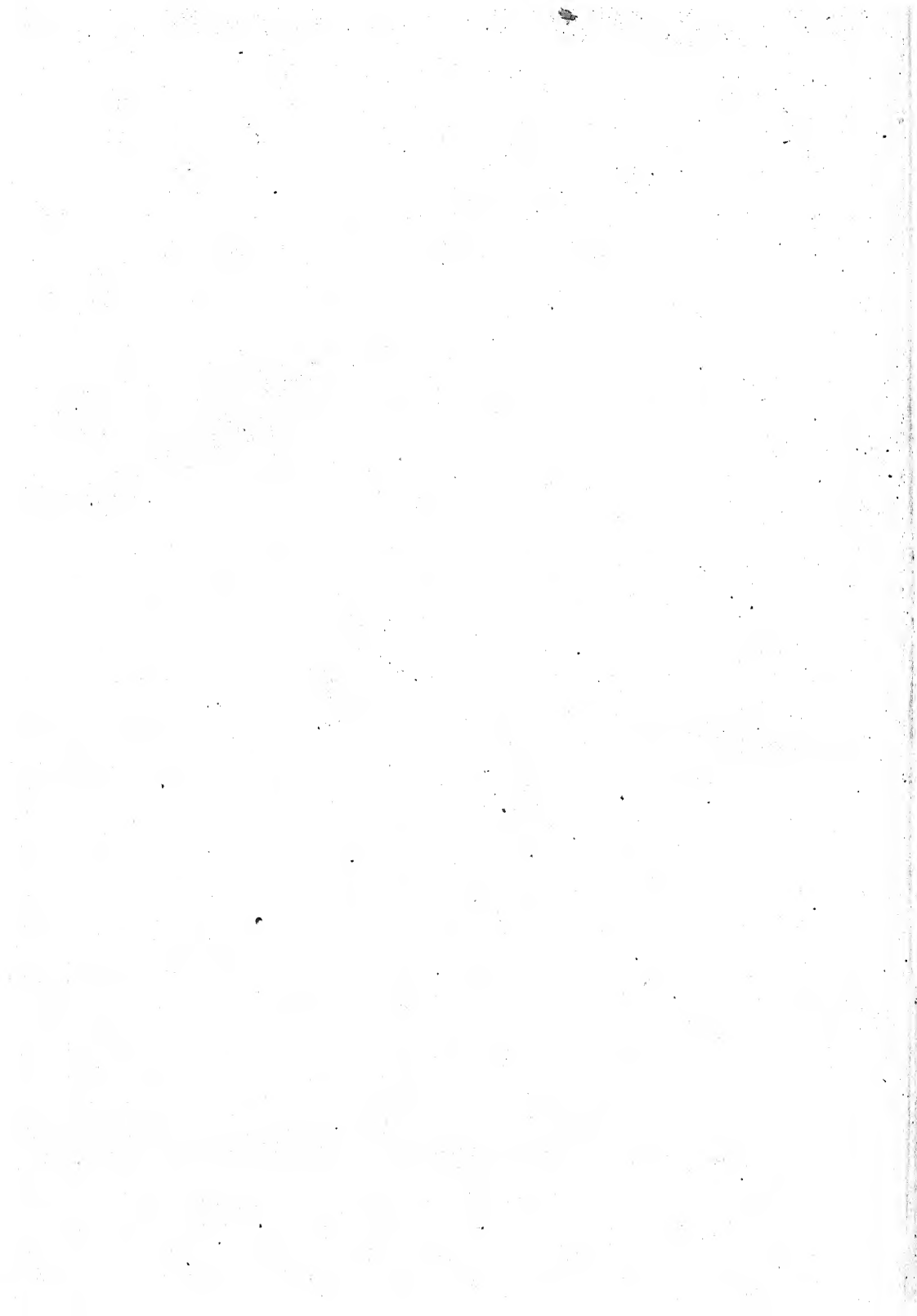Figs. 11, 12. Two autosomal fragments in 25-type male. Fig. 11. First metaphase. Fig. 12. First anaphase.

PLATE 13
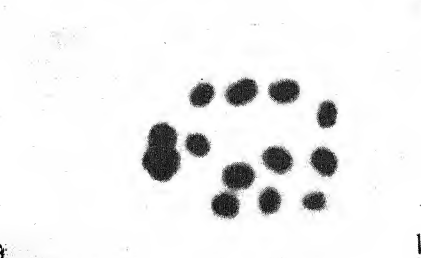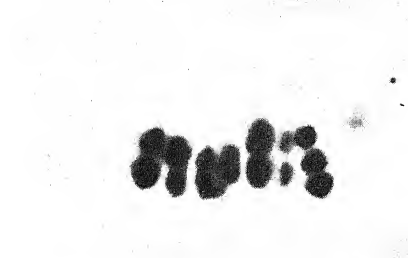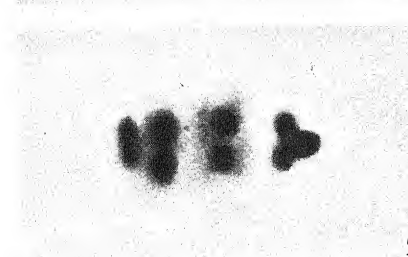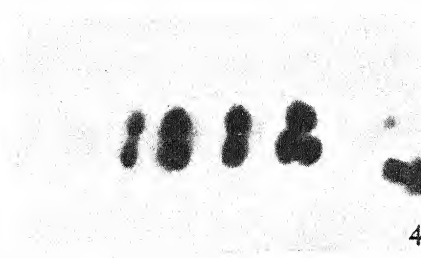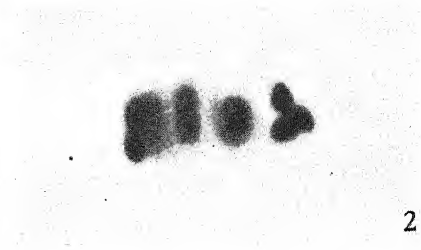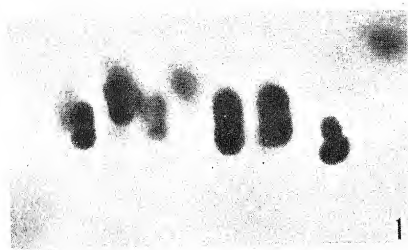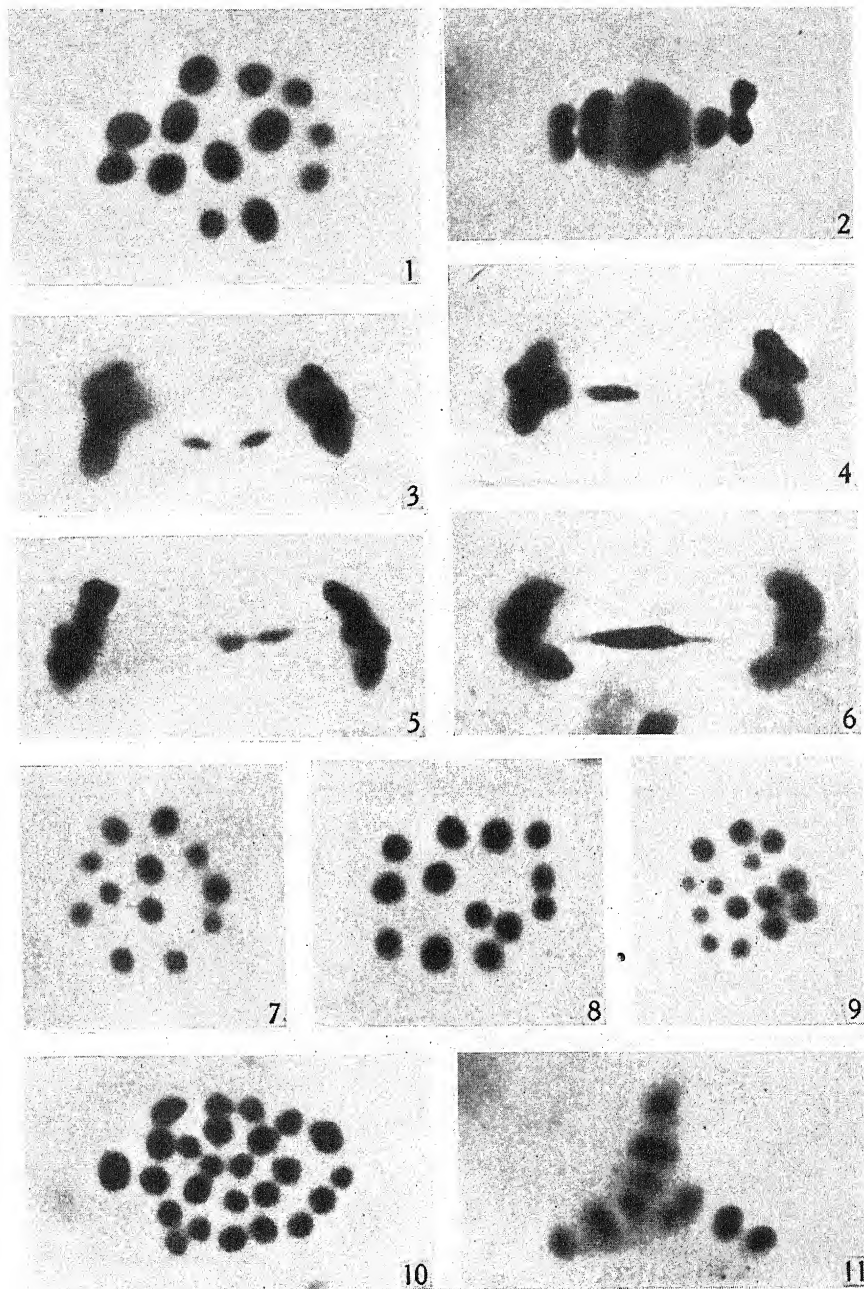
THE GENETICAL AND MECHANICAL PROPERTIES OF THE SEX CHROMOSOMES

VII. *APODEMUS SYLVATICUS* AND *A. HEBRIDENSIS*

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(With Nine Text-figures)

INTRODUCTION

A STUDY was made of the chromosome complements in the male sex of two species of *Apodemus*, *A. sylvaticus*, the common field-mouse, which inhabits the mainland of Britain and is also commonly found in Central Europe (Brohmer, 1929; Heinrich, 1929), and *A. hebridensis*, which is restricted in its distribution to the Outer Hebrides, off the north-west coast of Scotland (Koller, 1939).[1] Besides the specific morphological differences, such as coat colour, size, etc.; a difference was detected in the sex-determining mechanism. The structural peculiarities of the sex chromosomes in *A. sylvaticus* and in various other species excluding *A. hebridensis* have been described by several cytologists (cf. Oguma, 1934; Tateishi, 1934, 1935; Matthey, 1936a, b, 1938; and Raynaud, 1936), and attempts have been made to interpret the behaviour of the sex-determining mechanism during meiosis. These interpretations are based upon assumptions which are contradictory not only to the interpretation put forward by the present author to explain the genetical and mechanical properties of sex chromosomes in various mammals (mouse, rat, golden hamster, mole, ferret, squirrel, marsupials, man), but also to those principles which are shown, by the most extensive cytogenetical investigations, to govern chromosome behaviour in general. Because of the repeated appearance of these erroneous conceptions (see Minouchi, 1928; Oguma, 1934, 1937; Matthey, 1936a, b, 1938), a correction was felt to be imperative; hence the present paper, besides describing the structural differentiation in the sex chromosomes of *A. sylvaticus* and *A. hebridensis*, will deal in general with the controversial subject of the structure and behaviour of the morphologically unequal sex chromosomes.

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[1] The author is greatly indebted to Mr T. Warwick, of the Royal (Dick) Veterinary College, Edinburgh, for kindly supplying him with the material.

STRUCTURE AND BEHAVIOUR OF THE SEX CHROMOSOMES
DURING MEIOSIS AND MITOSIS

The diploid chromosome number in the male sex is 48 in both species (Fig. 1 *a*, *b*). While the same number was counted in *A. sylvaticus*, *A. flavicollis*, *A. agrarius* (Matthey, 1936*b*; Raynaud, 1936), *A. semotus*, *A. agrarius ningpoensis*, and *A. speciosus speciosus* (Tateishi, 1934, 1935), Oguma (1934, 1937) claims that only 47 chromosomes are present in the spermatogonia of *A. speciosus ainu* and *A. geisha*. At mitotic metaphase the chromosomes show great variation in size; large, medium-sized and small chromosomes can be distinguished in both species under discussion. The length of the chromosomes varies between 5·5 and 1 μ. The position of the centromere is apparently nearly terminal because all the chromosomes are rod-shaped. An analysis of bivalent configurations during meiosis also shows that the loci of the centromeres are not terminal but
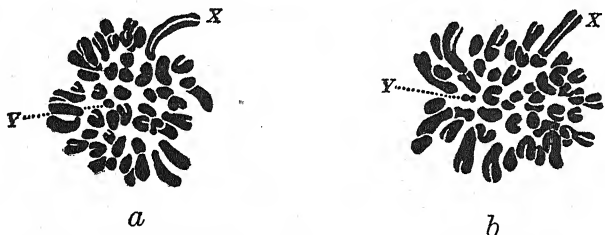

Fig. 1. Mitotic metaphase in *A. sylvaticus* (*a*) and in *A. hebridensis* (*b*). (× 3200.)

subterminal. Though the short arm may not be identifiable at mitotic metaphase, it is easily recognized in the larger chromosomes during meiosis. Contrary to the author's observations, Matthey (1938) is of the opinion that the majority of the chromosomes in *A. sylvaticus* as well as in *A. agrarius* are "telomitic" or telocentric, having a terminal centromere. Recent observations concerning the function and structure of the centromere (Darlington, 1939*a*, 1940) have shown that telocentric chromosomes are the result of misdivision of the centromere and, as a consequence of this, exhibit abnormalities which lead to their elimination in the course of evolution. Erroneous interpretations of mammalian chromosome structure are due to their great variability, attributable only to fixation. The primary constrictions, which are assumed to represent the loci of the centromeres, are particularly affected by fixation. Descriptions of telocentric chromosomes are often given by those investigators who have satisfied themselves with a limited number

of observations and have failed to compare chromosome structure with chromosome behaviour during mitosis and meiosis (Matthey, 1938).

An analysis of chromosomes in the diploid complex of *A. sylvaticus* and *A. hebridensis* has shown that the smallest and one of the largest chromosomes have no corresponding partners. This unequal chromosome pair is assumed to represent the sex chromosomes, *X* and *Y*. It was furthermore discovered that the two species differ in respect of the *Y*-chromosome, it being at least twice as large in *A. sylvaticus* as in *A. hebridensis* (Fig. 1 *a*, *b*). This appears to be the only detectable difference in the chromosome morphology of the two species.

In both species, during the leptotene and the zygotene stages of meiosis, a nucleolus-like structure can be distinguished as a permanent constituent of the nucleus. Its shape and size vary and it contains two regions, one stained deeply and the other lightly.

By following the behaviour of this structure in successive stages of meiosis, it was ascertained that it is the "sex-chromosome nucleolus", and that it represents the two associated and precociously condensed sex chromosomes. The structural differentiation of the sex-chromosome nucleolus is clearly discernible during the growth period of the meiotic prophase, when the autosomal bivalents temporarily lose the ability of staining, but the sex chromosome nucleolus stains and shows its double structure (Fig. 2). No difference was observed in the form and behaviour of the sex chromosome nucleolus in *A. sylvaticus* and *A. hebridensis* during the prophase of meiosis.

At the first meiotic metaphase in both species the *XY* complex has a well-differentiated structure; it is composed of a deeply stained terminal region to which a thin, diffuse, lightly stained thread of varying thickness is attached (Figs. 3, 4). The size of these two regions of the *XY* varies in different primary spermatocytes, indicating a time-lag in the behaviour of the different parts of the sex bivalent. As mentioned previously, the *X*- and *Y*-chromosomes are represented by an unequal chromosome pair in the diploid chromosome complement, and hence during meiosis they necessarily form an unequal bivalent which is made up of a pairing and a differential segment. Such bivalents are easily recognizable on account of their asymmetrical shape, and have been reported by various investigators in widely different organisms (Robertson, 1916; Wenrich, 1916; Carothers, 1926, 1931). But it is known now that unequal chromosomes may form an apparently "symmetrical" bivalent; the shape of the metaphase bivalent is determined by the position of the pairing segment in relation to the centromere and differential segment
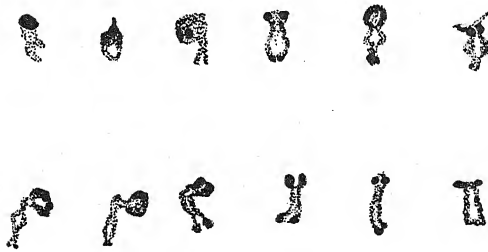
Fig. 2. Various configurations of the precociously condensed sex-chromosome during the growth period of meiotic prophase. (× 3200).
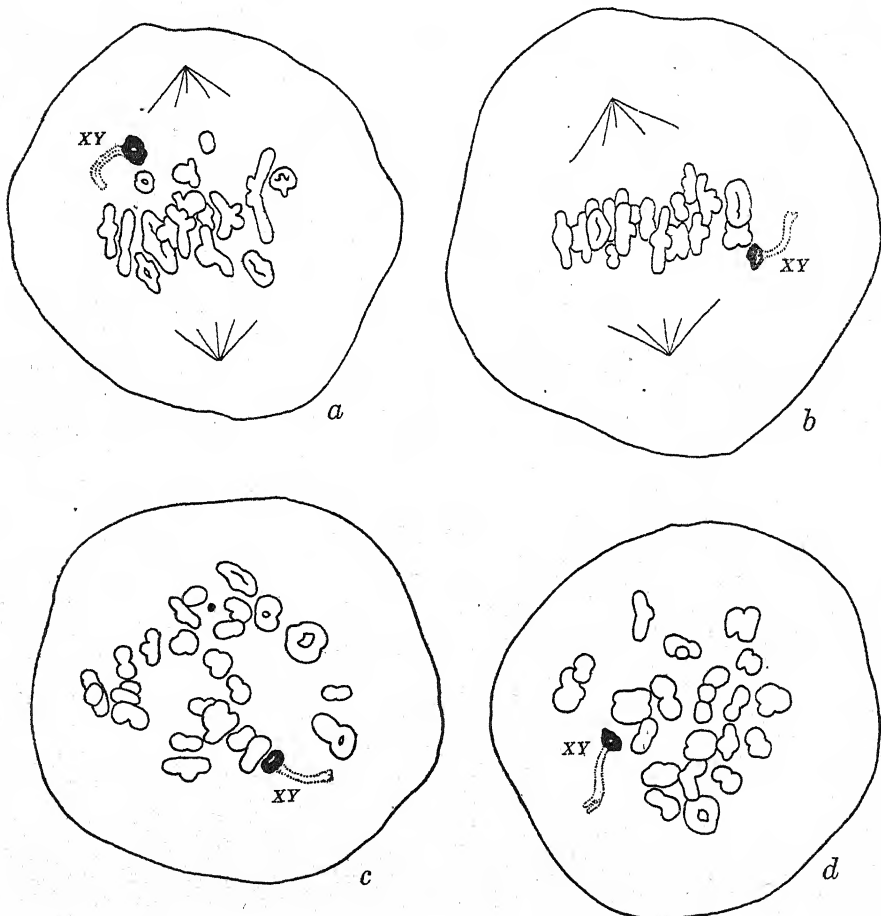

Fig. 3. Side view (*a*, *b*) and polar view (*c*, *d*) of first meiotic metaphase in *A. sylvaticus*, showing the deeply stained and diffuse regions of the sex bivalent. (× 3200.)
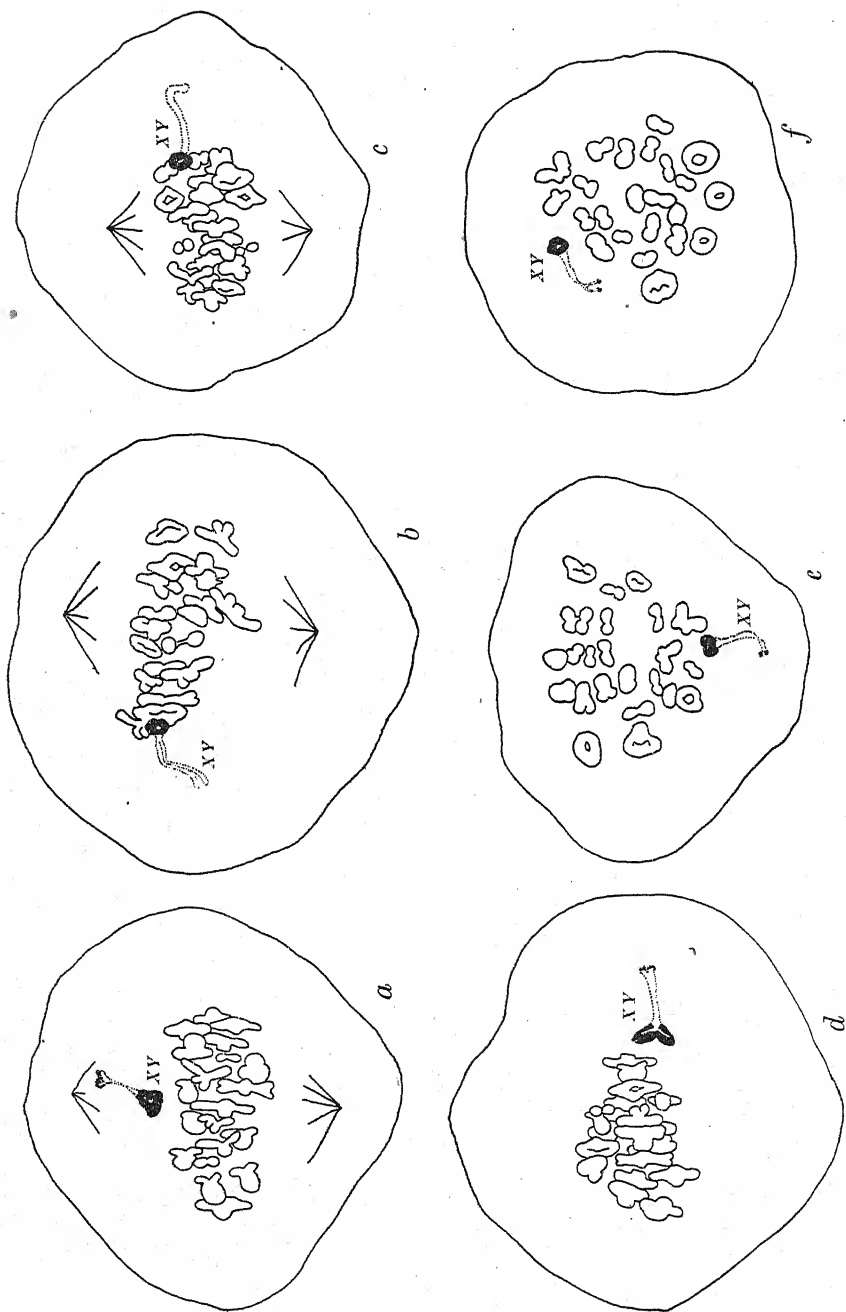
Fig. 4. Side view (a, b, c, d) and polar view (e, f) of first meiotic metaphase in *A. hebridensis*. The sex bivalent is very similar in form to that in *A. sylvaticus*. (×3200.)

(Koller & Darlington, 1934). The *XY* bivalent of *A. sylvaticus* and *A. hebridensis* closely resembles the symmetrical sex bivalent, first observed in the rat (Koller & Darlington, 1934) and later identified in several other organisms (Koller, 1937).
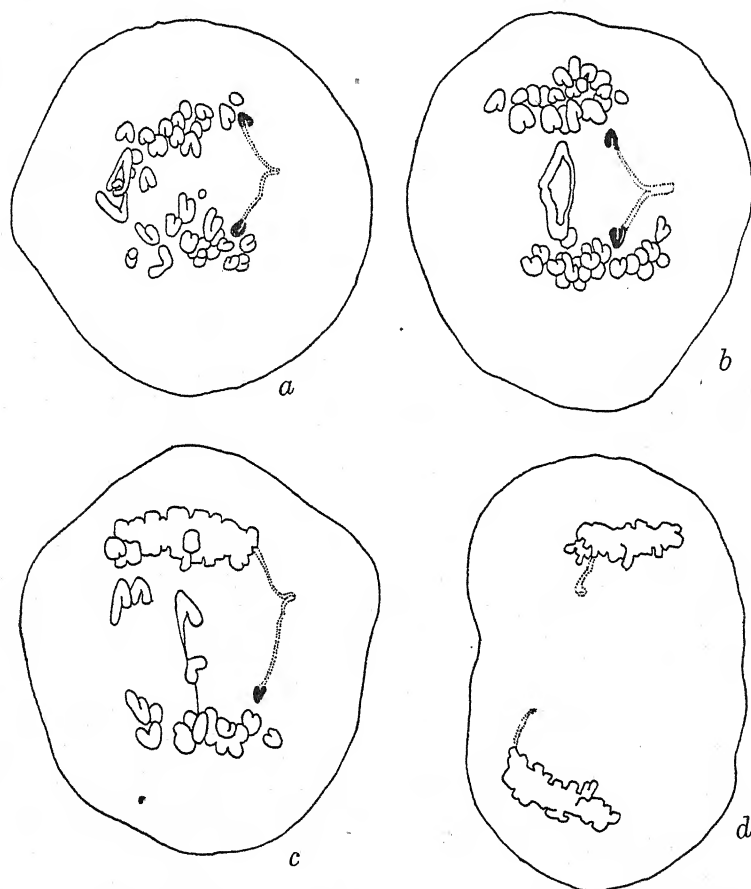
Fig. 5. First (*a*, *b*) and second (*c*, *d*) meiotic anaphase in *A. hebridensis*. The segregation of the *X*- and *Y*-chromosomes is invariably post-reductional. (× 3200.)

Though the configuration of the *XY* bivalent at meiotic metaphase is very similar in both species, the method of segregation of the *X*- and *Y*-chromosomes during first meiotic anaphase is different. In *A. hebridensis* the sex bivalent always divides equationally at the first anaphase (Fig. 5), and reduction of the *X* and *Y* takes place at the second meiotic anaphase; in *A. sylvaticus* about 8% of the primary spermatocytes show

reductional segregation of the X and Y during the first anaphase (Figs. 6, 7), i.e. while pre-reduction of X and Y is facultative in *A. sylvaticus*, it is obligatory in *A. hebridensis*. The behaviour of the
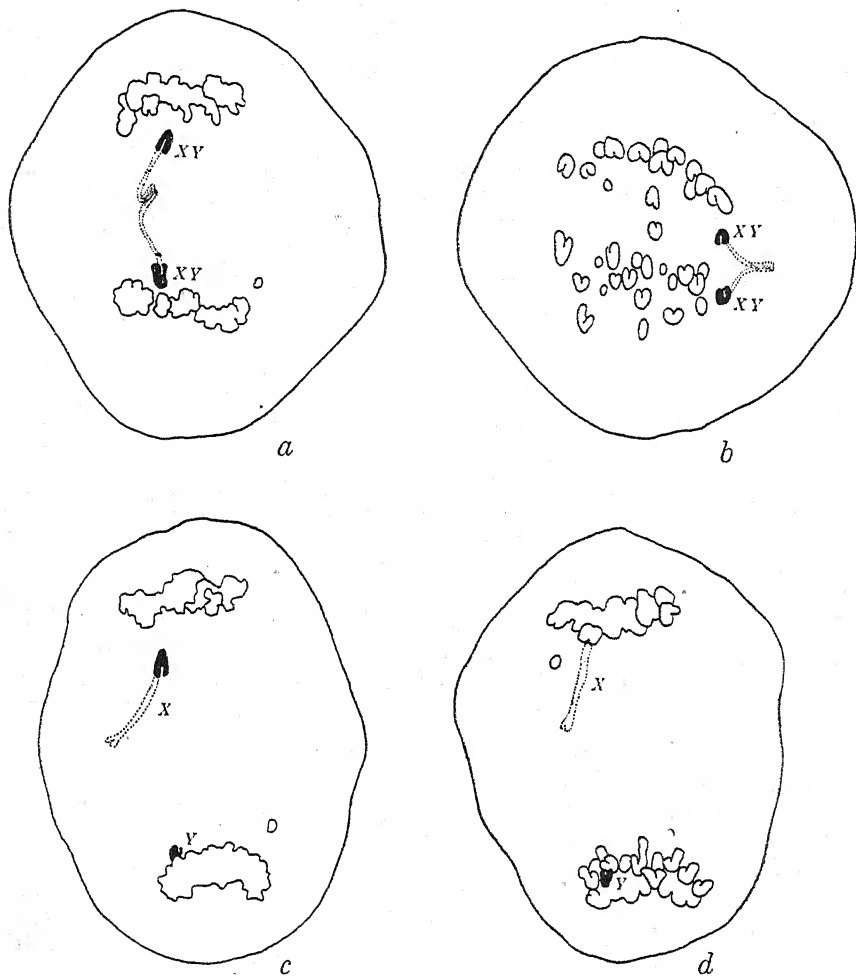
Fig. 6. First meiotic anaphase in *A. sylvaticus* showing post-reductional (*a, b*) and pre-reductional (*c, d*) segregation of X- and Y-chromosomes. ($\times 3200$.)

sex bivalents in *A. agrarius* and *A. flavicollis* and *A. agrarius ningpoensis* is similar to that observed in *A. sylvaticus*. It is interesting to note that no visible differences were detected in the configuration of the pre- and the post-reductional sex bivalents of *A. sylvaticus*. This observation

clearly indicates that an unequal chromosome pair may form bivalents which are similar in appearance but different in internal structure.

The behaviour of sex bivalents at meiotic metaphase and anaphase enables us to determine the structure of these sex chromosomes. It is
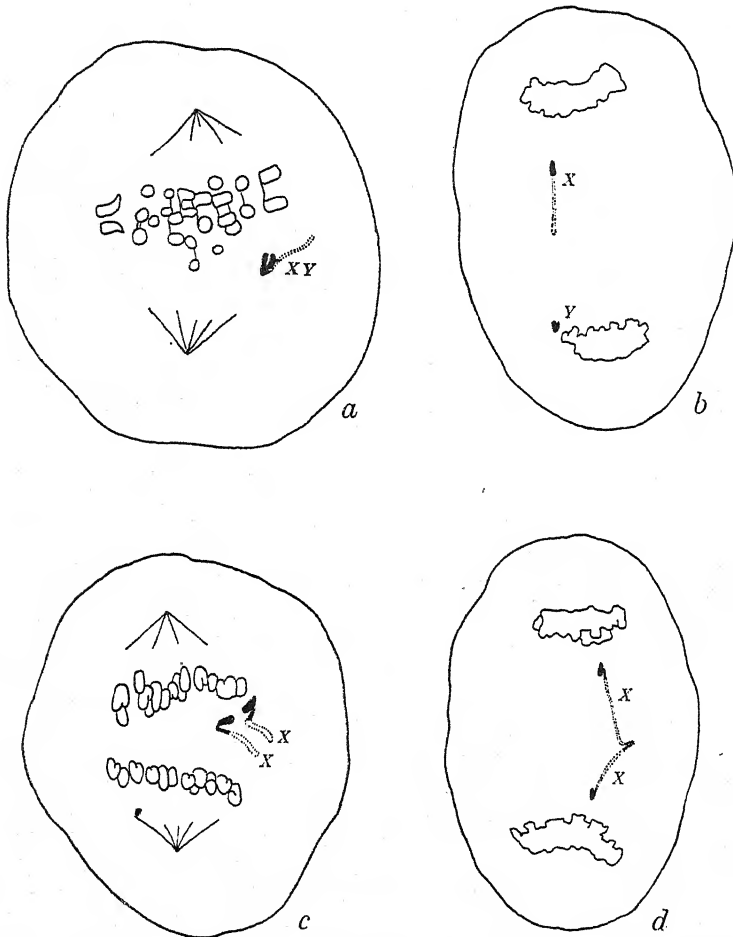
Fig. 7. Second meiotic anaphase and telophase in *A. sylvaticus*, showing post-reduction (*a*, *b*) and pre-reduction (*c*, *d*) of the *X*- and *Y*-chromosomes. (× 3200.)

accepted that metaphase association of chromosomes and their segregation at anaphase are normally conditioned by chiasmata formed during meiotic prophase (cf. Darlington, 1937). It has already been demonstrated that the sex chromosomes (with a few exceptions) are subject to the same mechanism of chromosome pairing and post-pachytene

association as other chromosomes (Koller, 1937). A structurally unequal chromosome pair such as the X and Y of $A.$ *sylvaticus* and $A.$ *hebridensis* is divided into a pairing and a non-pairing or differential segment. Chiasmata are formed only in the pairing segment, the differential segment having no partner. The position of the pairing segment in relation to the centromere and to the differential segment is the factor which determines the meiotic behaviour, particularly the pre- or post-reductional segregation of the unequal chromosomes. The existence of both kinds of segregation in $A.$ *sylvaticus* indicates that the centromere is interstitial and that chiasmata may be formed on either side. The pairing segment is divided by the centromere into a distal (or outer) and a proximal (or inner) portion; the latter is proximal, the former distal, to the differential segment. Crossing-over or chiasma formation in the outer pairing segment leads to pre-reductional segregation, while crossing-over in the inner pairing segment is responsible for the post-reduction of the X and Y. Segregation of one type only indicates the presence of one pairing segment. Thus, the complete lack of pre-reduction of X and Y in $A.$ *hebridensis* suggests that only the proximal pairing segment is present in the X and Y, or that, if a distal pairing segment is present, it is so short that no chiasma is ever formed in it. When both kinds of segregation of X and Y are encountered during the first meiotic anaphase, the relative lengths of the distal and proximal pairing segments may be estimated by the frequencies of pre- or post-reduction in the primary spermatocytes. In $A.$ *sylvaticus* 8 % of these show pre-reduction and 92 % post-reduction; consequently we may infer that the distal pairing segment is very short compared with the proximal one. In $A.$ *sylvaticus* the apparently similar configuration of the pre- and post-reductional sex bivalents during metaphase may be explained by assuming that the repulsion which normally operates between pairs of homologous regions lapses in the proximal pairing segment of the X and Y. It is suggested that the distal region of the Y, though no chiasma is formed in this segment, remains joined to the corresponding region of the X-chromosome. This similarity of the pre- and post-reductional XY bivalent during metaphase is responsible for the erroneous interpretations which have been put forward by several investigators (see Matthey, 1938). A detailed analysis of the behaviour of the XY bivalent during the various stages of meiosis, however, enables us to reconstruct the internal differentiation of the sex chromosomes in $A.$ *sylvaticus* and $A.$ *hebridensis*, and this is illustrated in Figs. 8 and 9.

DISCUSSION

It can be seen from the description given above that the sex chromosomes in *Apodemus* are subject to the laws which govern chromosome behaviour in general. The variations seen in the behaviour of the X and Y are only secondary results of the internal and external differentiation
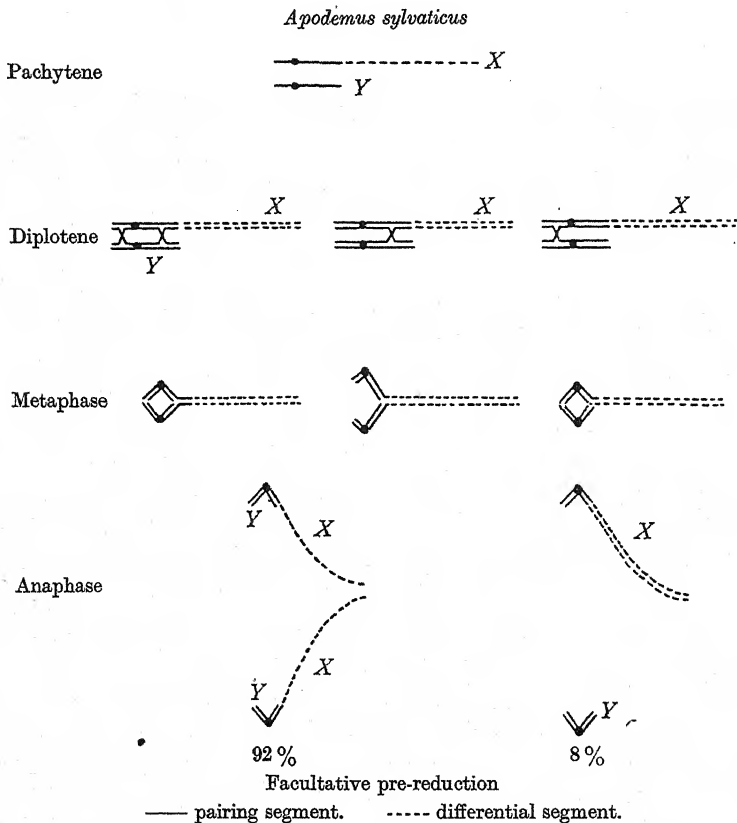
Fig. 8. Diagram illustrating the structure of the sex chromosomes in *Apodemus sylvaticus*. The pairing segment is divided by the centromere into a distal (outer) and a proximal (inner) region.

which occurred in these chromosomes. The changes in time relationship in the different regions of the sex chromosomes, expressed by precocity and heteropycnosis, result from a qualitative differentiation, while the size difference between the sex chromosomes is due to structural differentiation. It is shown that their segregation at meiosis depends on metaphase association which is conditioned by chiasma formation or

genetical crossing-over in the regions which are represented in the X- and Y-chromosomes. During the meiotic prophase and metaphase the position and number of chiasmata in the sex bivalent of *Apodemus* are obscured because of its peculiar properties resulting from qualitative differentiation. The segregation of X- and Y-chromosomes, however, can easily be followed at the first meiotic anaphase in various *Apodemus*

Apodemus hebridensis
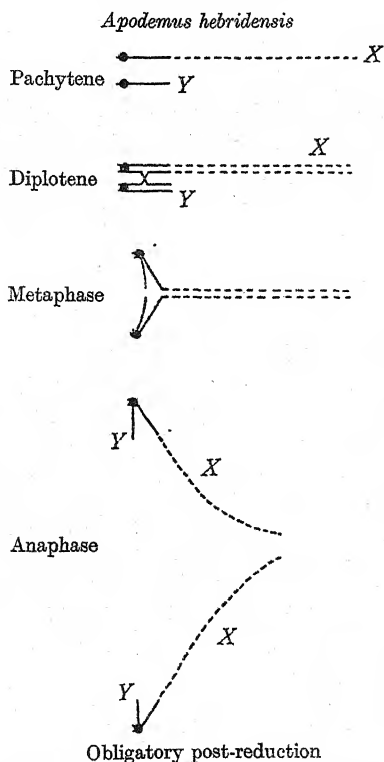
Fig. 9. Diagram illustrating the structure of the sex chromosomes in *Apodemus hebridensis*. The distal pairing segment is either absent or too small for chiasma to have formed.

species and has already been reported by several investigators (Matthey, 1936*a*, *b*; Oguma, 1934; Raynaud, 1936; Tateishi, 1935). Matthey alone made an attempt to explain how post- and pre-reductional segregation of the X and Y can occur in *A. sylvaticus* and *A. agrarius* (Matthey, 1936*a*, *b*, 1938), assuming that post-reduction is brought about by crossing-over between the paired X- and Y-chromosomes, while pre-reduction is due to lack of crossing-over. Other erroneous conceptions of Matthey are the association of non-homologous regions in the chromo-

somes, crossing-over of sister chromatids, and a temporary inactivity of the centromere during the first meiotic anaphase. The analysis of chromosome behaviour given above, however, has shown that there is no justification for attributing such peculiar properties to the sex chromosomes in order to explain the metaphase configuration and anaphase segregation of the X and Y in *Apodemus*.

It has already been mentioned that the diploid chromosome number in the male of various *Apodemus* species is 48, except in the two Japanese species, *A. speciosus ainu* and *A. geisha*, in which Oguma counted 47 chromosomes. According to him, in *A. speciosus ainu* the Y-chromosome is absent and the single X always divides equationally during the first meiotic anaphase. The configuration of the sex complex in this species during the first meiotic metaphase is similar to that found in *A. sylvaticus* and *A. hebridensis*, and it is more than probable that this represents not a single X but the XY bivalent. Tateishi (1935), in another Japanese species, *A. agrarius ningpoensis*, observed a chromosome configuration exactly similar to that found in *A. speciosus ainu*, which he identified as the XY bivalent. The only evidence which favours Oguma's assumption that the heterogametic sex in *A. speciosus ainu* and *A. geisha* is of the X-O type is that he actually counted 47 chromosomes in the spermatogonial division. It is, however, very probable that a Y-chromosome is present but is too small to be discerned during mitotic metaphase in the spermatogonia. This point has already been stressed by Matthey (1938), who also favours the view that *A. speciosus ainu* and *A. agrarius ningpoensis* are probably the same species.

The various *Apodemus* species may be arranged in the following series, according to the size of the Y-chromosome: *A. speciosus ainu*, with the smallest Y, *A. agrarius ningpoensis*, *A. geisha*, *A. speciosus speciosus*, *A. hebridensis*, *A. agrarius*, *A. flavicollis* and *A. sylvaticus*. The question may be asked how such differences in the size of the Y-chromosome of closely related species came about. It may be assumed that the differentiation of the sex chromosomes is a gradual evolutionary process, in which the following steps may occur: (1) the origin, by mutation, of a gene pair, or sex differentials; the segregation of these genes determines the two sexes; (2) suppression of crossing-over in the region where the sex differentials are localized; the suppression is genotypically controlled; (3) the development of the differential segment through structural changes in the region which contains the sex differentials.

The evolution of the sex chromosomes depends primarily on the

genetic history of the differential segments (Darlington, 1939b). In the homogametic sex, the differential segment of the X may cross-over while in the heterogametic sex the Y is excluded from crossing-over and consequently the size of the differential segment may alter not only as between related species but frequently within the same species. Thus the great variation observed in the size of the Y-chromosome in the various *Apodemus* species may have been brought about by suppression of crossing-over in the differential segment. To explain the difference in the method of segregation of the sex chromosomes of *A. hebridensis* and *A. sylvaticus* during the first meiotic anaphase, one must assume that another divergence has taken place, namely a change in the position of the centromere, which is located interstitially in the pairing segment. The change in the position of the centromere is brought about by a pericentric inversion (Muller, 1940) which includes the centromere; the distal breakage point is near the end of the chromosome, while the proximal is next to the centromere. This structural change will lead to the reduction of the distal or outer pairing segment to such an extent that it may be represented by only a very few genes, and crossing-over will be absent; hence a post-reduction of the X- and Y-chromosomes will be obligatory. We may visualize the differences in the structure and behaviour of the X- and Y-chromosomes in *A. sylvaticus* and *A. hebridensis* as being due to secondary structural changes, one of which involves the differential and the other, the pairing segment. It can be seen that an analysis of the structure and behaviour of X and Y in various species of *Apodemus* enables us to gain an insight into those processes through which the sex-determining mechanism has evolved.

SUMMARY

1. The diploid chromosome number in the spermatogonia of *Apodemus sylvaticus* and *A. hebridensis* is 48.

2. The sex chromosomes are unequal in size; the X is the largest chromosome in the complement, the Y is the smallest. The Y chromosome is smaller in *A. hebridensis* than in *A. sylvaticus*.

3. The sex bivalent shows a uniform symmetrical configuration during meiotic metaphase in both species.

4. In *A. hebridensis* post-reduction is obligatory, the X and Y segregate at the second meiotic anaphase. In *A. sylvaticus* 8% of the primary spermatocytes show pre-reduction, and 92% post-reduction, of the X and Y.

5. The behaviour of the X- and Y-chromosomes of *A. sylvaticus* during meiosis suggests that the pairing segment is composed of a distal and a proximal region, i.e. that the centromere is located interstitially and chiasmata can be formed on either side of it.

6. In *A. hebridensis* the behaviour of the XY bivalent suggests that the distal pairing segment is either entirely absent or is too small for chiasma to have formed, and hence segregation of the X and Y is always post-reductional.

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THE GENETICS OF BLACKARM RESISTANCE

II. CLASSIFICATION, ON THEIR RESISTANCE, OF COTTON TYPES AND STRAINS

III. INHERITANCE IN CROSSES WITHIN THE *GOSSYPIUM HIRSUTUM* GROUP

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(With Three Text-figures)

CONTENTS

Introduction

In an earlier paper the authors (1939) discussed the genetics of blackarm resistance in an American × Sakel[1] cross and the transference of the factors for resistance from the American to the Sakel genotype. The work described in the present paper was started before that on the transference of resistance to Sakel types, but had to be subordinated to the latter owing to the extreme urgency of this problem.

Now that the production of blackarm-resistant Sakels is nearing completion, the problem of adding resistance to American Upland strains will again come to the fore.

In the Southern Sudan, American Upland cotton is grown under rain conditions and, often, on the same site for several years. Clearly this present lack of rotation favours the spread of the disease, and under such conditions blackarm is likely to develop into a major problem as it has in Uganda (Hansford, 1934). Indeed, it seems certain that it would have done so already were it not for the smallness and isolation of most of the plots of cotton in the rain areas. Moreover, seed disinfection and plant hygiene as methods of blackarm control, although partially successful in the Gezira,[2] will not be practicable in the Southern Sudan for many years to come.

It is clear, therefore, that the only satisfactory approach to the problem of combating this disease in the more inaccessible parts of the Sudan is by the breeding of resistant types. A necessary approach to this problem was an examination of the blackarm resistance potentialities within the genus *Gossypium*. Part II of this series gives a classification, based on resistance, of a wide range of cotton types, whilst an account of the inheritance of resistance in some of the crosses which have been undertaken is given in Part III.

Previous work

Knight & Clouston (1939) briefly described a technique for infecting cotton plants with blackarm on a standardized basis by spraying with a water suspension of the causal organism (*Bact. malvacearum* Sm.) obtained from infected plant material. A system of grading blackarm resistance, with grade "0" representing immunity and "12" full susceptibility, was also defined in detail.

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[1] Sakel, an abbreviation for Sakellaridis, is a variety of *G. barbadense*. American Upland cottons belong to the species *G. hirsutum*.

[2] The Gezira is an area lying between the Blue Nile and the White Nile and irrigated from the Sennar dam.

In the same paper it was shown that the resistance of the American Upland (*G. hirsutum*) strain, Uganda B. 31, is due to the presence of two dominant, cumulative factors, B_1 and B_2 (together with a modifier complex). These factors, when transferred to a Sakel (*G. barbadense*) genotype, gave grade "10. 1" and "7" resistance respectively and clearly defined ratios were obtained in F_2 and in backcross progenies on the expected basis for a two-factor difference.

II. CLASSIFICATION, ON THEIR RESISTANCE, OF COTTON TYPES AND STRAINS

The resistance of Uganda B. 31 is marked, but, though the transference of B. 31 resistance factors to Sakel affords considerable protection against blackarm, it was desirable to discover whether other factors for resistance existed, with a view to their ultimate incorporation in Sakel and American varieties.

With this end in view, seed of a large number of cotton types was sown in one plot, the varieties being plentifully interspersed with controls of known blackarm grade. Six weeks after sowing, all the plants were sprayed with blackarm suspension and, after a suitable incubation period, graded for resistance. The results of this examination are given below. Such a list, including as it does over one hundred and sixty varieties and strains of cotton, should be of direct use to a plant breeder suddenly faced with the necessity of breeding cotton varieties resistant to this disease. It must be remembered, however, that this classification is based solely on leaf attack and that no account is taken of the ability or otherwise of the plant to "grow away" from the disease. Tissue resistance is the criterion and not the quality of vigour which may enable a plant to recover from an attack—this latter is a separate problem. Stem resistance was not recorded, but the authors (1939) have shown that stem and leaf resistance are positively correlated in crosses involving factors B_1 and B_2, and observation indicates that this correlation is general.

Hansford (1934), writing of blackarm in Uganda, attributes the major damage to stem infection but states that this occurs in 99% of cases as the result of the extension of leaf attack and that, without the latter, infection of the stem will not occur. Uganda appears to differ, in this respect, from the Sudan where leaf infection alone is capable of considerably reducing the yield. It matters little, however, for plant breeding purposes, whether the crop loss is a direct or indirect sequel to leaf infection, since control of the latter still remains the key to the problem.

(1) *Peruvian group* (G. barbadense *Linn.*)

(*a*) *Sakels.* Some forty strains and substrains of Sakel have been tested and all showed full susceptibility (grade "12" symptoms).

(*b*) *"Egyptian" types.* Sixteen types have been examined, namely, "310", NT. 25/24, Nahdah, Garofallou, Pima, Nazli, and Giza nos. 3, 7, 12, 15, 19, 24, 25, 26, 27, 28. All these proved fully susceptible except that Giza nos. 7 and 12 might have contained a very slight degree of resistance—a difference too slight to be measured on the "0"–"12" scale.

(*c*) *Pure Sea Island and strains of Sea Island origin.* Thirteen strains were examined: D. 3. 2, D. 1. 8, D. 14. 1, NT. 4/33, "897", "898", AN, NT. 12/34, NT. 13/34, and Shambur nos. III and IV; all showed grade "12" symptoms. V. 135 and "Montserrat" were graded as slightly under "12"—a degree of resistance so slight that place effect could readily account for it. In addition, a single plant was found in "898" showing grade "7" resistance.[1]

(*d*) *Ishan.* Ishan A is fully susceptible.

(*e*) *Hybrids.* The following types were found to be fully susceptible:

K. 3 × S. 5. 39 (Kidney × Sea Island)
K. 3 × S. 5. 58 (Kidney × Sea Island)
K. 3 × S. 5. 124* (Kidney × Sea Island)
K. 3 × S. 1. 74* (Kidney × Sea Island)
K. 3 × S. 1. 99 (Kidney × Sea Island)
K. 3 × S. 1. 107 (Kidney × Sea Island)
K. 3 × S. 1. 118* (Kidney × Sea Island)
K. 3 × S. 1. 160 (Kidney × Sea Island)
K. 3 × S. 1. 238* (Kidney × Sea Island)
Red Sea Island (Kidney × Sea Island)
NT. 5/33 (Afifi × Burd's Sea Island)
508 B (Sea Island × Sakel)
NT. 8/34 (Peruvian × Sea Island)
NT. 40/36 (Ishan × Sakel)
NT. 37/36 (Ishan × Sakel)

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[1] A paper on the genetics of the resistance of this plant is in the course of preparation by the senior author.

* In the following classification an asterisk signifies that less than ten plants were examined.

(2) *American Upland group* (G. hirsutum *Linn.*)

Sixty-six strains and substrains were examined. They were classified first according to whether they were pure breeding for the degree of resistance shown, and secondly, on average blackarm grade.

(a) *Types showing little or no variation in blackarm symptoms.*

(i) *Blackarm grade* "9"–"10". Delta Webber 6, Webber nos. 38 and 49. 2, Uganda S.G. nos. 26, 27 and 29*, Willett's Red Leaf, Rustam 82*, 514, 514 A, 514 B, 514 D, 514 E, N.T. 6/34*, Dekhan 169, Batyr 508, Ak Dzhura, Schroeder 1306*, Columbia 44*, Wild's no. 1, Bolland Upland nos. 29, 99*, and 105*, XA 129*, XA 1129, Triumph nos. 145. C. 51. 30*, 143. J. 4. 119, and 143. K. 2. 130*, NT. 60/39, NT. 68/39, NT. 75/39 and NT. 77/39.

(ii) *Blackarm grade* "7"–"8". Meade*.

(iii) *Blackarm grade* "5"–"6". 513, 513 A, 513 B, 513 E, N.T. 58/39, NT. 61/39, NT. 84/39, Parnell's U. 4. Nyasa 5, NT. 15/35, 511 A, 511 C, 511 E, Triumph 145. C. 52. 32*, NT. 7/34.

(iv) *Blackarm grade* "3"–"4". Uganda B. 31/21, Uganda B. 31/10/12/11.

(b) *Types impure for blackarm resistance.*

Type	Modal grade	Range
Rustam 124*	"9"	"7"– "9"
Columbia 40	"11"	"9"–"12"
Columbia 43	"9"	"9"–"11"
Delrect I	"10"	"7"–"10"
Parnell's U. 4*	"5"	"5"– "7"
Parnell's U. 4. 4. 2	"5"	"5"–"11"
Parnell's U. 4. Nyasa 4	?	"5"– "9"
Parnell's U. 4. Nyasa 8	?	"5"–"10"
Parnell's U. 4. Nyasa 9	"6"	"5"– "9"
Parnell's U. 4. Nyasa 10	"6"	"5"– "9"
511	"6"	"6"–"10"
Pump Scheme Strain	"10"	"7"–"12"
NT. 74/39	"5"	"4"–"10"
NT. 63/38	?	"4"–"12"
NT. 69/39	"5"	"5"–"10"
NT. 78/39	"9"–"10"	"5"–"10"
NT. 83/39	"5"– "6"	"5"– "8"

(3) *Bourbon group* (G. purpurascens *Poir.*)

"Serido" and "Moco" were fully susceptible but the sample of the latter contained one grade "10" plant.

(4) *Punctatum group* (G. punctatum *Sch. & Thon.*)

"Gambia native" and XBA 22039 possessed grade "1"–"2" resistance whilst samples of "Hindi Weed" (? *G. punctatum*) from the

commercial Sakel crop were pure for grade "5"–"6" resistance where they had not outcrossed with Sakel.

(5) *Interspecific hybrids and other types*

Type	Modal grade	Range
Verdao* (American Upland × Brazil perennial)	"12"	—
R.U. 4 (36) 23–1 (Asiatic × New World)	"3"	—
Titsiros	"12"	"10"–"12"
Kawa Baladi	"9"	"9"–"10"

(6) *Old World* (13 *chromosome*) *cottons*

Five types were tested: Abu Hareira, *G. cernuum* and *G. sanguineum* proved immune, "Chinese Indigenous" gave a range of grades from "0" to "9", whilst Nuba Red was pure breeding for grade "9".

Discussion

In the American Upland group resistance ranged, in pure types, from grade "3" to "10", whilst some of the types impure for resistance contained fully susceptible plants. It would appear that grade "9"–"10" is the normal "full susceptibility" in American varieties, and this probably represents the grade "12" of *G. barbadense* modified by such factors as hairiness, rapid maturity and hardening of leaves. Recent work by the senior author indicates the probability that B_2 is the major factor responsible for the resistance of most of the American strains of grade "7" and under.

From the results obtained, the Bourbon group appears to contain little, if any, resistance, but a much more varied sample would be required to determine the resistance potentialities of this group with any certainty. Harland (1939), writing of *purpurascens* types obtained from Serido, noted that "One type was so badly attacked that it was almost impossible to carry the plants past the seedling stage, and the susceptibility was fully inherited in the next generation. Another type proved to be practically immune". This statement is particularly interesting because of the strong suggestion of seed infection as a factor determining the severity of attack on the first variety. In view of the fact that these Brazil types are often heterozygous for fuzzy seededness, it is possible that the suggestions made below with regard to the St Vincent results might also explain Harland's findings.

In the Punctatum group, Gambia native and XBA 22039 (a derivative of an "off-type" plant found in "Schroeder") show outstanding resistance and this is the strongest resistance so far found in New World types.

No indication of blackarm resistance was discovered in the Peruvian group with the exception of the single (? mutant) plant referred to in paragraph 1 (c). Other workers, however, have claimed the existence of marked resistance in *G. barbadense*. Lambert (1938) noted the remarkable power of recovery from blackarm possessed by the X 1530 and X 1730 types as compared with ordinary Sakel. This, however, is due to the greater vigour of the former which enables the plant to "grow away" from the disease, an effect which can be observed in ordinary Sakel growing under good conditions.

Bailey (1928, 1929) recorded varying "susceptibility" on six Egyptian types under test and noted the same order of varietal susceptibility at each of three stations[1] within the same year. In the following season, the results from the three stations again agreed closely, but the order of susceptibility of the six varieties differed markedly from that of the previous season. In view of the inconsistency of the results of the two years' work the intervarietal differences noted could not be attributed to genetic resistance and Bailey therefore suggested that the varying attack might be due to differential seed infection. As the seed supply of these varieties came from districts known to differ markedly in blackarm severity, this assumption seems reasonable. Under standard conditions these varieties have since been shown to be equally and fully susceptible.

This illustrates the divergent results which might be obtained in a search for true resistance in the absence of standardized inoculation and provides a possible explanation of the failure to find, at Shambat, the resistance claimed by Burd, Harland and Evelyn for certain Sea Island strains and hybrids.

Burd (1925), working in St Vincent, described the Sea Island strain "AN" as "somewhat resistant to angular leaf spot", whilst Harland & Evelyn (1933) considered that all their pedigree cultures possessed a "considerable degree of resistance". In the following year Evelyn & Harland (1934) reported: "A striking feature of the Red Sea Island strains...is their apparent immunity to Angular Leaf Spot" and again "with the exception of the Moco strains, Ishan and the hybrid Ishan × V. 135, the strains can be regarded as being highly resistant, particularly the Red Sea Island strains which appear to be completely immune." Finally, Evelyn (1936) wrote: "The Red Sea Island strains are in general highly resistant to Angular Leaf Spot."

Of the strains handled by these workers "AN" and Red Sea Island proved fully susceptible at Shambat, whilst V. 135 showed a degree of

[1] Shambat, near Khartoum, and Wad Medani and Barakat in the Gezira.

resistance so slight that place effect could readily account for it. On the other hand, Evelyn (1937) recorded his Asiatic × New World strains as being highly resistant, and these strains tested at Shambat did show considerable resistance (para. 5).

It is a matter of some importance to know whether blackarm resistance factors may operate in one place and not in another. The lack of agreement between the St Vincent and Shambat results therefore deserves consideration. From the published reports of the St Vincent workers it would appear that the incidence of blackarm, as measured by the percentage infection of the crop, was the criterion on which varietal resistance was based. Thus Evelyn & Harland (1934) stated that, for angular leaf spot, counts were made at weekly intervals, plants attacked being marked to avoid re-examination. As these observations were made on plants not subjected to any standard infection but which contracted the disease naturally, it seems highly probable that factors other than, or in addition to, true tissue resistance were operating. As already stated plant vigour is an important factor in blackarm incidence and, as Bailey (1929) showed, comparison of intervarietal attack can be readily vitiated by the degree of primary infection of the respective seed lots used. Some such effect might explain the divergence between the St Vincent and Shambat results. In this connexion it is interesting to note that the strains found to be susceptible in St Vincent are, in general, those with most fuzz on the seed, e.g. Moco, Ishan and to a lesser extent Montserrat, which Harland (1939) described as "rather susceptible". Obviously seed fuzz provides a foothold for the bacterial slime, and the amount of primary infection[1] should be positively correlated with the degree of fuzziness.

Experience in the Sudan has clearly shown the supreme importance of seed infection, and though seed disinfection is not absolutely effective it has removed the risk of complete crop failure which previously obtained in this country as a result of early blackarm attack. It seems, therefore, that the resistance obtained in St Vincent might be due almost entirely to absence of seed fuzz with the concomitant reduction of primary infection. Where, however, true resistance occurs (e.g. in the Asiatic × New World types) the presence or absence of fuzz would be relatively unimportant. Moreover, judged on Sudan results, the very vigorous growth obtained in St Vincent would accentuate differences due to differential seed infection, whilst the heavy rainfall, during the

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[1] Primary infection is the attack on the cotyledons resulting from *B. malvacearum* carried on the seed coat or fuzz.

"dead" season for cotton, would largely inactivate blackarm debris, thereby further accentuating the importance of primary infection. In this connexion it is noteworthy that Squire (1938), working on the survival of pink bollworm, stressed the rapidity of disintegration of cotton debris in the West Indies.

III. Inheritance in crosses within the Gossypium hirsutum group

(1) Uganda B. 31 ×514 crosses

The object of this cross was to transfer, by backcrossing to the 514 parent, the blackarm resistance of Uganda B. 31.

514 was bred in the Sudan from a selection made in Pump Scheme Strain and is an erect, "open", rather tall type with good quality lint. This strain possesses only very slight (grade "10") blackarm resistance, and it was thought that any increase in this resistance would be of marked value in the rain areas.

Uganda B. 31, when grown at Shambat, is a short, dark green type of very unattractive appearance and low yielding capacity. The leaves are extremely susceptible to attack by sucking insects. The main stem internodes are condensed and the sympodia very short. In the Southern Sudan, however, B. 31 has a very different appearance: the internodes are longer, the leaves are healthier and the type is not unattractive but the yield is poor. The value of B. 31 lies in its very marked (grade "3") blackarm resistance.

F₁ of Uganda B. 31× 514.

In 1934–5 season a number of F_1 families were grown, these being the result of crossing Uganda B. 31 with substrains of 514. At this stage no standard system of infecting the plants nor of grading for resistance had been evolved.

The F_1 plants, together with the parent types, were sprayed with blackarm suspension 7 weeks after sowing and were later examined for the disease after an adequate incubation period had elapsed. The severity of attack was found to be intermediate between the marked resistance of B. 31 and the susceptibility of 514, though there was a range from true intermediacy down to resistance as marked as that of B. 31, due, undoubtedly, to the inaccuracy of infection and grading referred to.

In subsequent seasons the sowing and grading of this F_1 and of the parent types was repeated. With the knowledge of spraying gained by

experience and working on a more perfectly defined system of grading, a much more even result was obtained. B. 31 was found to have an average blackarm grade of "3" with a range from "2" to an occasional "4" and 514 was graded steadily between "9" and "10" whilst the F_1 was graded at "6" with little variation.

First backcross to 514.

In 1935–6 season the first backcross to 514 was grown, sprayed with blackarm inoculum 6 weeks after sowing and later graded. The summarized results of this grading are tabulated below, and graphed in Fig. 1.

Table 1. *Summation of blackarm classification of first backcross to 514*

	Blackarm grade												
	"1"	"2"	"3"	"4"	"5"	"6"	"7"	"8"	"9"	"10"	"11"	"12"	Total
	—	25	82	170	33	10	35	88	129	37	—	—	609
Percentages	—	4·1	13·5	27·9	5·4	1·6	5·7	14·4	21·2	6·1	—	—	99·9
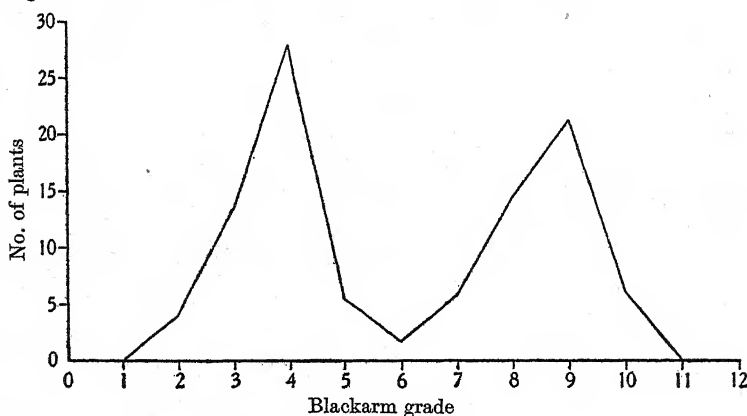
Fig. 1. (Uganda B. 31 × 514) × 514.

These figures give very little information as to the factorial nature of resistance, but, if the frequencies are split into two groups omitting grade "6" (the point of minimum frequency), the ratio of 289 less-resistant to 310 more-resistant plants is obtained and this hints at a 1 : 1 ratio.

F_2 of Uganda B. 31 × 514.

In 1935–6 season a number of F_2 families were grown. These were sprayed with inoculum and graded.

Normal spacing for cotton at Shambat is 50 × 90 cm., and, where blackarm grading is to be done, sowing is carried out at one seed per

hole or else the plants are thinned at a very early stage to one plant per hole. The 1935–6 F_2 families shown in Table 2, however, were sown at 25×90 cm. spacing, with up to four plants per hole according to the amount of seed available. This was done because, though it was necessary to have a large population to work with, considerations of available land and labour precluded the adoption of normal spacing.

When blackarm grading was carried out it became obvious that this overcrowding must have affected the efficacy of spraying and it seemed likely that the shade effects produced by overcrowding might also have vitiated the results (Knight, 1935). A second large F_2 was, therefore, grown at normal spacing in 1936–7 to check the previous season's data. The two seasons' results are compared in Table 2 and those for the latter

Table 2. *Summation of blackarm classification of F_2 of Uganda B. 31 × 514. Comparison of 1935–6 with 1936–7 results*

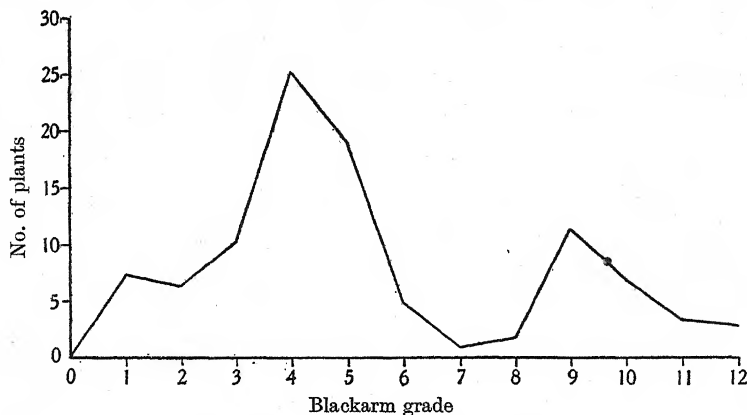|	Blackarm grade												
	"1"	"2"	"3"	"4"	"5"	"6"	"7"	"8"	"9"	"10"	"11"	"12"	Totals
Distribution													
1935–6	173	91	140	171	159	72	46	24	57	61	57	17	1068
1936–7	183	156	256	632	475	119	22	42	284	172	83	70	2494
Percentages													
1935–6	16·3	8·6	12·4	16·1	15·0	6·8	4·3	2·3	5·4	5·8	5·4	1·6	100·0
1936–7	7·3	6·3	10·3	25·3	19·0	4·8	0·9	1·7	11·4	6·9	3·3	2·8	100·0

Fig. 2. Uganda B. 31 × 514 F_2.

season are graphed in Fig. 2, the earlier results being omitted on account of the considerations stated above.

A comparison of the two seasons' results shows that the normal spacing has brought about a drift from grades "1"–"3" into grades "4" and "5" and from "6"–"8" into grade "9". It is unlikely that this

change was due to climate as the grading standard in each season was based on numerous controls of the parent types. These results further stress the absolute necessity for complete standardization in this work.

A number of markedly dwarfed[1] plants, about 8 in. high, appeared in F_2 and also in the F_2 of the first backcross to 514. This dwarfing has been found to be due to complimentary factors, and it is closely linked

Table 3. *Summation of blackarm classification of F_2*
of first backcross to 514

	Blackarm grade												
	"1"	"2"	"3"	"4"	"5"	"6"	"7"	"8"	"9"	"10"	"11"	"12"	Total
	139	139	195	781	949	410	141	193	386	96	60	31	3520
Percen- tages	3·9	3·9	5·5	22·2	27·0	11·6	4·1	5·5	11·0	2·7	1·7	0·9	100·0
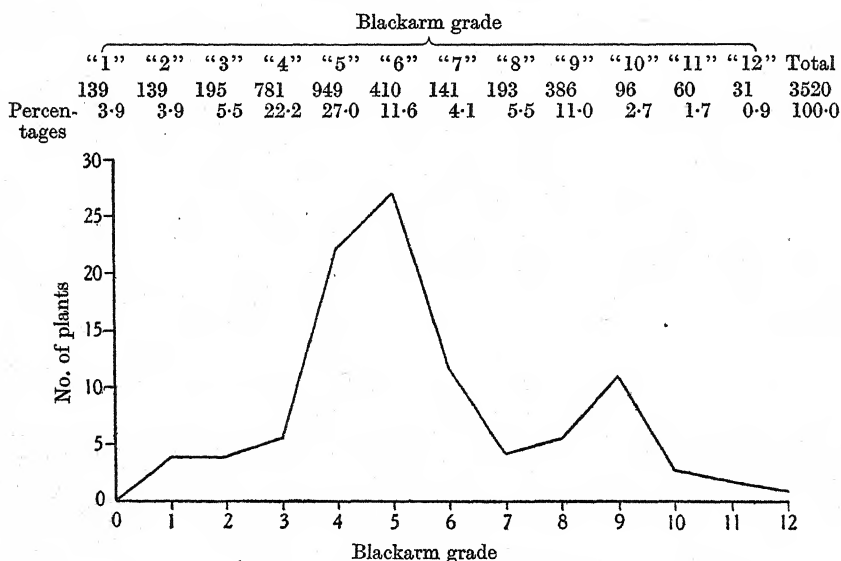
Fig. 3. (Uganda B. 31 × 514) × 514 F_2.

with factor $\mathbf{B}_1$. These dwarfs do not occur in all American Upland crosses involving Uganda B. 31, nor do they appear in crosses between this strain and Sakel.

F_2 of first backcross to 514.

All plants in the first backcross showing grade "4" or less infection were reselected in the field for nearness to 514 appearance and, later, in the laboratory, for lint quality and ginning out-turn. Plants of grade "3" and "4" only remained after this rigorous selection. Self-bred seed of these was sown in 1936–7 season and, 6 weeks after sowing, the plants were sprayed with blackarm suspension and, later, graded. The grading of all these families is summarized in Table 3 and graphed in Fig. 3.

[1] A paper on the genetics of this character is in course of preparation by the senior author.

Discussion.

In crosses between Sakel and 514 (unpublished work) the F_1 was fully susceptible whilst, in F_2, resistance ranged, with no clear-cut groups, from the grade "10" of the 514 parent to the full susceptibility of Sakel. This suggests that several minor factors are responsible for 514 resistance and that these are in the main recessive.

It is known that Uganda B. 31 contains two blackarm resistance factors, B_1 and B_2, and in crosses between this variety and Sakel, clear-cut ratios of three resistant to one fully susceptible occur in the first and second Sakel backcrosses. With the almost complete removal of the B. 31 modifier complex in the third and later backcrosses, ratios of 2 : 1 : 1 of the following composition were obtained (Knight & Clouston, 1939):

		Grade in 5th Sakel backcross
Phenotype A 1	$\begin{cases} \mathbf{B_1}\ \mathbf{b_1}\ \mathbf{B_2}\ \mathbf{b_2} \\ \mathbf{b_1}\ \mathbf{b_1}\ \mathbf{B_2}\ \mathbf{b_2} \end{cases}$	"7" + "6" + "5"
Phenotype B 1	$\mathbf{B_1}\ \mathbf{b_1}\ \mathbf{b_2}\ \mathbf{b_2}$	"10. 1"
Phenotype C 1	$\mathbf{b_1}\ \mathbf{b_1}\ \mathbf{b_2}\ \mathbf{b_2}$	"12"

Crosses between Sakel heterozygous for factor B_1 and 514 yield, in F_1, a 1 : 1 ratio of grades "10. 1" to "12" (unpublished work), grade "10. 1" being the type of resistance produced by B_1 in the Sakel genotype. On the other hand, 514 to which factor B_2 has been transferred is graded as "5" though B_2 gives grade "7" resistance in a Sakel leaf (NT. 61/39 in para. 2 (*a* iii) of Part II is of this type and though classified in the grade "5"–"6" group, this type produced no grade "6" lesions). The inference is that 514 contains no modifiers for factor B_1 but does possess modifiers for B_2.

514 was selected from Pump Scheme Strain which arose mainly from Nyasaland Upland (Bailey, 1927). The origin of Uganda B. 31 is obscure but it seems probable that it, also, arose from Nyasaland Upland. It is reasonable to assume, therefore, that some of the Uganda B. 31 resistance modifier complex is present in the 514 genotype. Thus, in the backcross (B. 31 × 514) × 514, the 3 : 1 ratio which obtains in early Sakel backcrosses would not be expected, since the absence of B_1 modifiers in 514 and the presence of B_2 modifiers would result in a widening of the gap between $B_1 b_2$ and $B_1 B_2$ or $b_1 B_2$ plants. It is suggested that the 1 : 1 ratio shown in Fig. 1 is made up of phenotype A (above) on the one side and B + C on the other and that B and C merge owing to the incorporation of a number of the 514 weak resistance factors. In this connexion it should be noted that "10. 1" resistance is slight so that phenotype B is

not far removed from C and any merging of the two would therefore readily account for the curve at the more susceptible end of the scale in Fig. 1.

Early Sakel backcrosses, involving Uganda B. 31, yield, in F_2, 15 : 1 ratios of resistant to fully susceptible plants whilst later ones give the expected ratio of 12 : 3 : 1. Though no 12 : 3 : 1 ratio is clearly defined in the F_2 of B. 31×514, Fig. 2 shows a basic distribution similar to this ratio and Fig. 3 confirms this. Fig. 3 tails off, at the susceptible end, more rapidly than Fig. 2, because of the inclusion of more weak 514 resistance in the first backcross F_2 than in the straight F_2. The subsidiary peak at grade "1" is partly due to the presence of dwarf types which showed an increased "resistance" owing to their shortened main stem and branch internodes, which rendered spraying of their leaves difficult.

(2a) *Uganda B. 31 × 513 crosses*

The object of this cross was to find out whether the resistance of 513 could be combined with that of Uganda B. 31 with the possibility of obtaining complete immunity to blackarm.

513 was selected in the Sudan from an importation of Punjab American Upland cotton. It is an early maturing, heavy yielding strain of medium quality. It is very hairy but requires rather frequent irrigation and is not very well suited to the Southern Sudan though it has proved of great value in the North. 513 is graded for blackarm attack as "5"–"6" at Shambat.

F_1 of Uganda B. 31 × 513.

In 1934–5 season a number of F_1 families were grown but, as was noted earlier, no standard system of infecting the plants nor of grading for resistance had been evolved at that stage so that the F_1 grade was not noted except that the blackarm symptoms were intermediate between Uganda B. 31 and 513.

First backcross to 513.

A number of first backcross families were sown in 1935–6, sprayed 6 weeks after sowing, and, later, graded (Table 4). As these families all gave similar results only the totals have been tabulated.

Table 4. *Summation of blackarm classification of first backcross to 513 parent*

Blackarm grade

"1"	"2"	"3"	"4"	"5"	"6"	"7"	"8"	"9"	"10"	"11"	"12"	Total
—	27	245	394	35	2	—	—	—	—	—	—	703

It is clear, from the absence of any fully susceptible types or, indeed, of any plants showing greater susceptibility than 513 (grade "5"–"6" resistance), that 513 and Uganda B. 31 have at least one resistance factor in common.

F_2 of Uganda B. 31×513.

The F_2 of Uganda B. 31×513 was grown in 1935–6 and gave the following distribution of blackarm grades.

Table 5. *Summation of blackarm classification of F_2*
of Uganda B. 31×513

Blackarm grade

"1"	"2"	"3"	"4"	"5"	"6"	"7"	"8"	"9"	"10"	"11"	"12"	Total
77	170	378	361	119	33	14	—	—	—	—	—	1152

A comparison of the distribution shown in the foregoing table with that given in Table 2 for the F_2 of Uganda B. 31×514 is instructive. It has been shown that 514 contains no major genes for resistance so the difference in distribution must be due to the resistance of 513, and again the absence of fully susceptible plants shows this resistance to be genetically identical with part of the resistance of Uganda B. 31.

F_2 of first backcross to 513.

A number of selections were made in the first backcross for breeding purposes. The seed of these was sown in 1936–7 season and a routine blackarm examination made later gave the following figures.

Table 6. *Blackarm classification of F_2 of (Uganda B. 31×513) $\times 513$*

Family no.	Grade of parent	"1"	"2"	"3"	"4"	"5"	"6"	"7"	"8"	"9"	"10"	"11"	"12"	Total
HA 12/36	"3"	—	—	5	64	21	—	—	—	—	—	—	—	90
HA 14/36	"3"	—	—	22	19	1	—	—	—	—	—	—	—	42
HA 15/36	"3"	—	1	27	54	11	—	—	—	—	—	—	—	93
HA 16/36	"3"	—	2	5	15	11	—	—	—	—	—	—	—	33
HA 19/36	"3"	—	7	13	11	10	—	—	—	—	—	—	—	41
HA 27/36	"3"	—	—	—	11	49	8	—	—	—	—	—	—	68
HA 10/36	"4"	—	—	1	33	45	1	—	—	—	—	—	—	80
HA 11/36	"4"	—	2	26	53	43	5	—	—	—	—	—	—	129
HA 17/36	"4"	—	—	—	5	31	—	—	—	—	—	—	—	36
HA 20/36	"4"	—	2	6	9	13	4	—	—	—	—	—	—	34
HA 22/36	"4"	—	—	—	24	36	13	1	—	—	—	—	—	74
HA 23/36	"4"	—	—	2	12	96	48	1	—	—	—	—	—	159
HA 24/36	"4"	—	—	—	21	31	1	—	—	—	—	—	—	53
HA 25/36	"4"	—	—	9	19	51	2	—	—	—	—	—	—	81
HA 13/36	"5"	—	—	—	5	28	5	—	—	—	—	—	—	38
Totals	"3"	—	10	72	174	103	8	—	—	—	—	—	—	367
Totals	"4"–"5"	—	4	44	181	374	79	2	—	—	—	—	—	684
Grand totals		—	14	116	355	477	87	2	—	—	—	—	—	1051

The distribution in families derived from parents of grade "3" resistance and those in families derived from grade "4"–"5" parents are totalled separately in Table 6. The value of selecting grade "3" plants is obvious, for their progenies have a mode at grade "4" whereas the modal point for the progenies of grade "4"–"5" plants is at grade "5". Had family HA 27/36 been omitted from these totals the difference would have been greater and it seems probable that the parent of this family was incorrectly graded.

(2b) *Crosses between 513 and Sakel*

The object of crossing 513 with Sakel was the transference of the 513 resistance into Sakel by repeated backcrossing. It was hoped that it might prove possible to add the resistance of 513 to that of the factors B_1 and B_2, the inclusion of which in the Sakel genotype is now accomplished.

F_1 of 513 × Sakel.

The resistance of the F_1 was similar to that of the 513 parent.

First Sakel backcross.

The grading of the first Sakel backcross was as follows.

Table 7. *Blackarm classification of first Sakel backcross*

Blackarm grade

"1"	"2"	"3"	"4"	"5"	"6"	"7"	"8"	"9"	"10"	"11"	"12"	Total
—	—	—	—	10	87	64	—	—	—	7	128	296
				161						135		
	Expected (1 : 1)			148						148		

$\chi^2 = 1.14$ and P is approximately 0.3.

If the grade "11" plants are included with the fully susceptible group a ratio of 161 resistant to 135 fully susceptible plants is obtained, and this is a fair approximation to a 1 : 1 ratio.

F_2 of 513 × Sakel.

An F_2 of 513 × Sakel was grown in the same season as the backcross, sprayed with inoculum 6 weeks after sowing and, later, graded with the following results.

Table 8. *Blackarm classification of F_2 of 513 × Sakel*

Blackarm grade

"1"	"2"	"3"	"4"	"5"	"6"	"7"	"8"	"9"	"10"	"11"	"12"	Total
—	—	—	9	134	210	137	5	8	6	36	61	606

Double F_1 (Sakel × Uganda B. 31) × (Sakel × 513).

In the hope of obtaining clearer results by eliminating a portion both of the B. 31 genotype and of the 513, a double F_1 was made by crossing the F_1 of Sakel × B. 31 with that of Sakel × 513. The results from the grading of this hybrid are tabulated below.

Table 9. *Blackarm classification of double F_1*
(Sakel × Uganda B. 31) × (Sakel × 513)

Blackarm grade

"1"	"2"	"3"	"4"	"5"	"6"	"7"	"8"	"9"	"10"	"11"	"12"	Total
—	2	2	14	37	65	60	—	3	29	3	31	246
				180					32		34	
	Expected (6 : 1 : 1)		184·4						30·8		30·8	

Discussion.

The absence, in the backcross (B. 31 × 513) × 513 (Table 4), of any plants even approaching full susceptibility indicates that Uganda B. 31 and 513 have at least one resistance factor in common. The F_2 of B. 31 × 513 (Table 5) bears out this conclusion and the grading of the F_2 families of the first backcross to 513 (Table 6) also agrees with the assumption that 513 and Uganda B. 31 share at least one resistance factor.

In crosses between Sakel and 513 the production of a 1 : 1 ratio in the first Sakel backcross (Table 7) indicates that 513 contains only one major factor for resistance. In the F_2 of Sakel × 513 (Table 8), however, the clear-cut 3 : 1 ratio which would be expected with a monofactorial difference is not obtained.

It is suggested that 513 contains the strong factor $\mathbf{B}_2$ together with a number of weak factors and that it is these weak factors which are responsible for the distribution which obtains in the F_2 of Sakel × 513 shown in Table 8.

If 513 contains $\mathbf{B}_2$ and no other major factors for resistance, then, in the double F_1 shown in Table 9, the following types should appear.

1 $\mathbf{B}_1$ $\mathbf{b}_1$ $\mathbf{B}_2$ $\mathbf{B}_2$ ⎫
2 $\mathbf{B}_1$ $\mathbf{b}_1$ $\mathbf{B}_2$ $\mathbf{b}_2$ ⎪ Grades "7" and under
1 $\mathbf{b}_1$ $\mathbf{b}_1$ $\mathbf{B}_2$ $\mathbf{B}_2$ ⎬
2 $\mathbf{b}_1$ $\mathbf{b}_1$ $\mathbf{B}_2$ $\mathbf{b}_2$ ⎭

1 $\mathbf{B}_1$ $\mathbf{b}_1$ $\mathbf{b}_2$ $\mathbf{b}_2$ Grade "10. 1" in Sakel but "10" and under in B. 31 × Sakel crosses, according to the proportion of B. 31 genotype

1 $\mathbf{b}_1$ $\mathbf{b}_1$ $\mathbf{b}_2$ $\mathbf{b}_2$ Grade "12" in Sakel

The agreement between expectation on this $6:1:1$ basis and the results tabulated in Table 9 is so close that there can be no doubt that the genetic make-up of 513, from the resistance standpoint, is as stated, and it is, therefore, impossible to combine the resistance of 513 with that of Uganda B. 31 in new strains. There is no point in adding the factor B_1 to the 513 genotype as, regardless of blackarm resistance, 513 is not well suited to rain cultivation in the Southern Sudan.

Summary

Part II of this series gives a classification, based on blackarm resistance, of over 160 varieties and strains of cotton. Complete immunity was not found in any New World types but exists in some of the Old World species. Several of the results disagreed with statements by St Vincent workers, some of the strains classed as "somewhat resistant", "highly resistant" and "immune" by them being found at Shambat to be fully susceptible. It is suggested that St Vincent results were based on differences in primary infection (seed infection), rather than on true tissue resistance and that these differences were accentuated by the presence or absence of seed fuzz and by plant vigour.

Part III shows the type of blackarm resistance inheritance obtaining in the crosses Uganda B. 31×514 and B. 31×513 (all three are American Upland types) the latter results being further clarified by crosses between 513 and Sakel.

Uganda B. 31 contains resistance factors B_1 and B_2 plus modifiers and shows grade "3" resistance; 514 contains no major resistance factors but numerous minor ones and is graded as "10" (grade "0" represents immunity and "12" full susceptibility). The first backcross to 514 gives a bimodal curve with peaks at grades "4" and "9" and displaying an approximate $1:1$ ratio instead of the $3:1$ ratio expected. In F_2 and in the F_2 of this first backcross, bimodal curves are again obtained, but these bear some resemblance to the $12:3:1$ ratio expected. The reasons for these anomalies are discussed.

It is shown that 513, a selection from a Punjab American Upland importation with grade "5"–"6" resistance, contains factor B_2 together with several very weak resistance factors.

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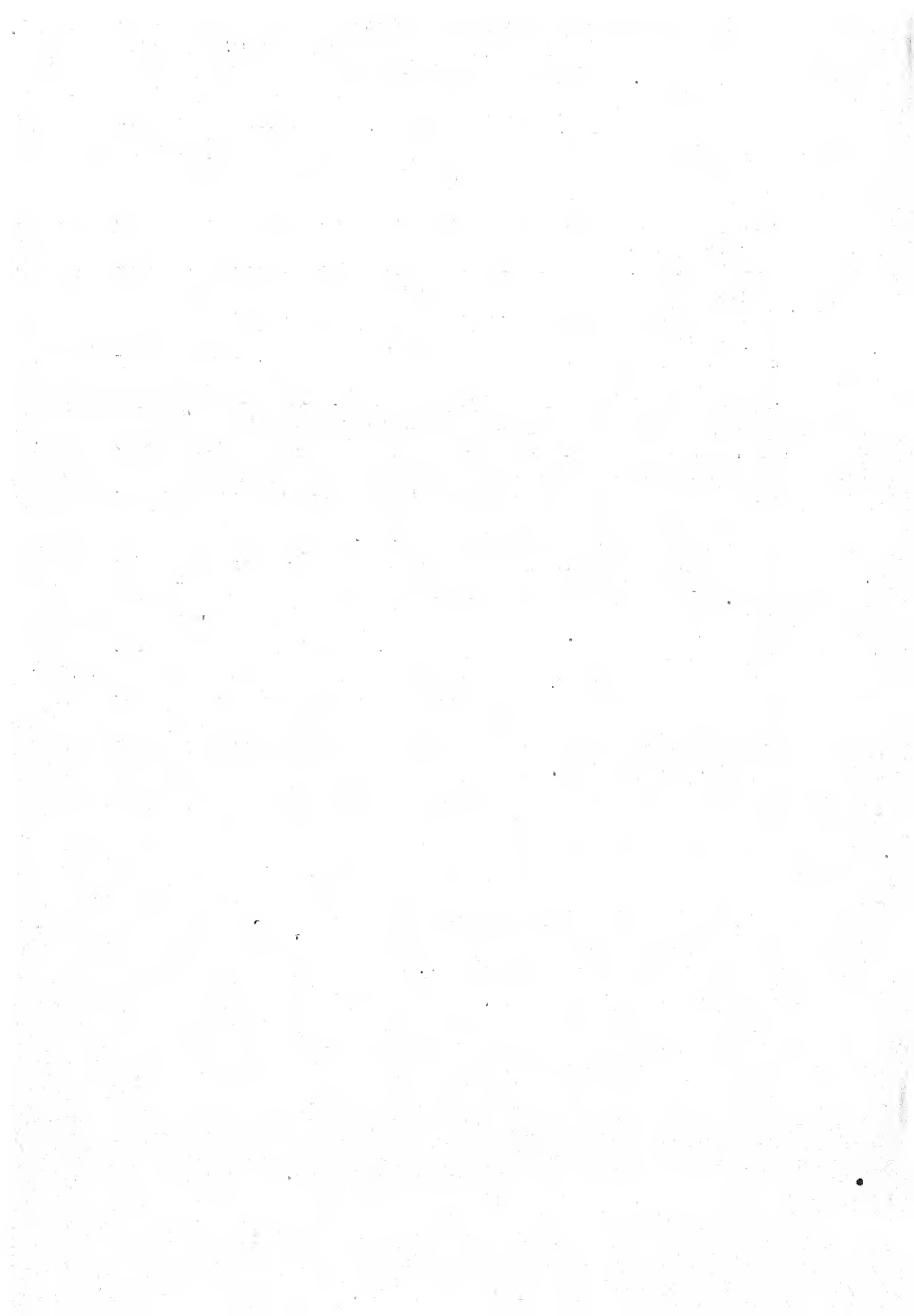
MEIOSIS IN DIPTERA. I

PROPHASE ASSOCIATIONS OF NON-HOMOLOGOUS CHROMO-
SOMES, AND THEIR RELATION TO MUTUAL ATTRACTION
BETWEEN CENTROMERES, CENTROSOMES AND CHROMO-
SOME ENDS

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(With Plate 14 and Twenty-five Text-figures)

CONTENTS

1. INTRODUCTION

MEIOSIS in male robber flies (Brachycera:Asilidae) was studied in the hope of throwing further light on the unusual type of meiosis which is characteristic of the males of higher Diptera, in which there is no chiasma formation or crossing-over between the homologous autosomes (Darlington, 1934). Previous work by Metz (1922) and Metz & Nonidez (1921, 1923, 1924) had indicated that Asilids would provide suitable material for this purpose.

During the course of this investigation it was found that association between the end and centromere regions of non-homologous chromosomes was a common occurrence, and was especially clear in *Habropogon appendiculatus* Schiner, which is the subject of this paper. In *Habropogon* the associations were subjected to both qualitative and quantitative analysis, and their development and dissolution traced. Their cause is discussed and the evidence for and against the hypothesis that the associations are due to mutual attractions between heterochromatic regions is analysed. It is concluded that the main cause of these and similar associations is probably mutual attraction between ends and centromeres, and, in certain circumstances, centrosomes.

2. Material and methods

Six male specimens of *H. appendiculatus* Schiner (Asilidae:Dasypogoninae) were collected at Pilat-Plage, Arcachon, France, on 8 August, 1939. They were dissected in their body fluids, and the testes transferred to four different fixatives: Medium Fleming, La Cour 2BE, Allen's Picro-Formol A3, and Craf, all made according to the formulae of La Cour (1937), but with the addition of 1% urea. The results produced by the three former fixatives were rather clearer than, but not essentially different from, those produced by the Craf. The preparations were mostly cut 14μ thick, and they were all stained by Newton's gentian-violet technique. The drawings were made with a camera lucida, and are reproduced at a magnification of 3200 diameters.

3. Spermatogonial divisions. The chromosome complement

The spermatogonial prophases are not well stained in my material, but one group of metaphase plates, of the fourth spermatogonial division, are deeply stained and very clear. Text-figs. 1 and 2 show two of these cells, each with five pairs of long chromosomes, and a small dot which represents the pair of sex chromosomes. The chromosomes show very clear secondary pairing between homologues.

Further information concerning the chromosome complement can be obtained by examining meiotic metaphase plates (Text-figs. 18, 19; Pl. 14, fig. 6), when it can be seen that all the autosomes have submedian centromeres, and that in three of them the two arms are of almost equal length, but in the longest one, and in one of the others, the arms are unequal in length. This difference sometimes enables them to be distinguished during prophase.

4. Meiotic prophase

In describing the prophase of meiosis in advanced Diptera difficulties of nomenclature arise because most of the descriptive names used for normal meiosis (leptotene, zygotene, diplotene, diakinesis) refer to characteristic stages which are not exactly paralleled in this material, owing to the presence of very strong secondary pairing and the absence of chiasma formation. Six stages can be clearly distinguished before
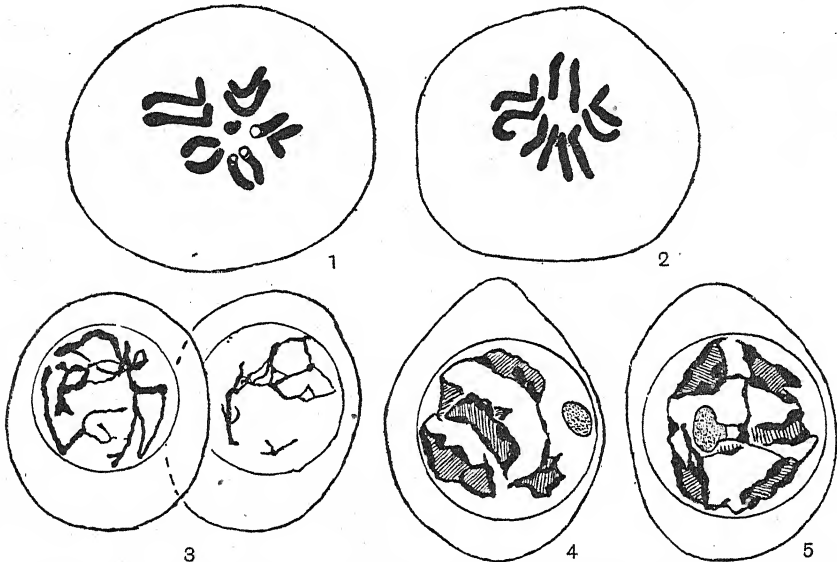
Text-figs. 1, 2. Fourth spermatogonial metaphase, showing somatic pairing. In Text-fig. 2 the sex chromosomes are shown off the plate, slightly above the autosomes.
Text-fig. 3. Stage A. Earliest prophase. Upper and lower views of the same cell.
Text-figs. 4, 5. Stage B. In Text-fig. 5 the sex chromosomes form a small deeply stained mass close to the nucleolus, and are attached to the nucleolus and to two autosome pairs.

metaphase, and they will be referred to as stages A–F, and their probable homology with the stages found in normal meiosis will be discussed later.

In *Habropogon appendiculatus* these stages may be described thus:

Stage A (Text-fig. 3). The continuation of spermatogonial telophase. No cell growth has occurred, and the nucleus contains, when fixed and stained, a number of fine threads which anastomose in various directions. Structure cannot be distinguished from artefact, but probably there are five thicker threads which represent the five pairs of autosomes. This stage is short, and passes into

Stage B (Text-figs. 4, 5). In stage B five large deeply stained irregular masses are present in the nucleus, and these are undoubtedly the five

pairs of autosomes. They are connected to each other, and also to a stained oval mass, the nucleolus, by fine threads which might either be artefacts or true connexions between non-homologues. Study of the
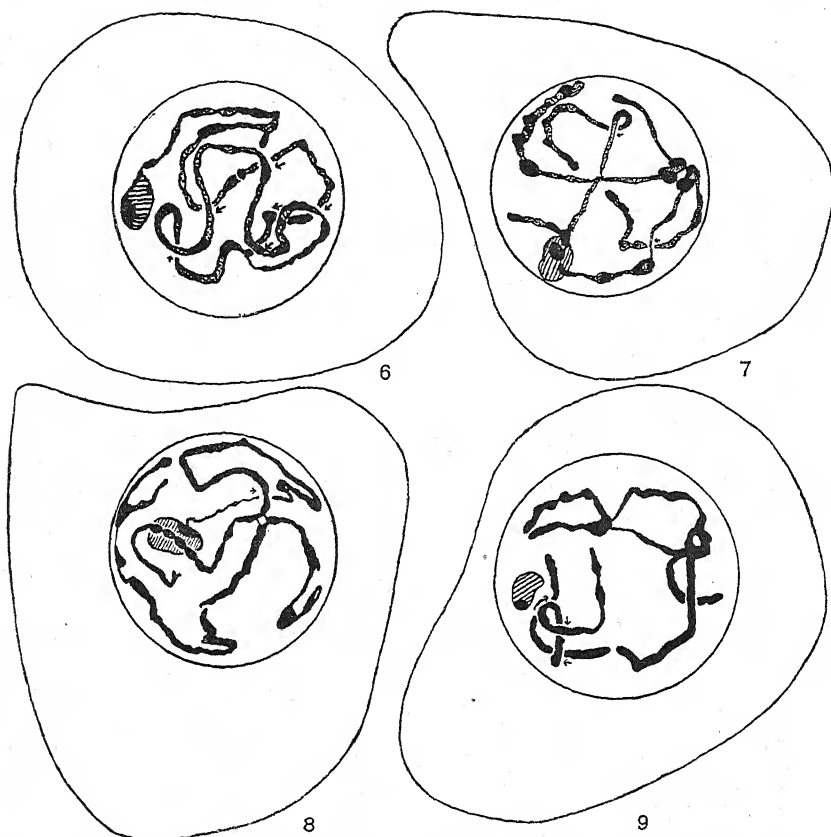
Text-fig. 6. Stage *C*. The centromere of the short unequal bivalent is associated with an end, and the short arm end of this bivalent is the only free end in the cell. It is situated at 1 o'clock.

Text-fig. 7. Stage C. In the centre is an association between a centromere and two ends, with no visible heterochromatin. At 3 o'clock there is an association of four ends. The sex chromosomes are associated with a centromere and an end.

Text-fig. 8. Stage D. An association between a centromere and two ends, and another between a centromere and one end.

Text-fig. 9. Stage D. The three bivalents involved in this configuration cannot be separately distinguished, but it is clear that one or other of the associations of four threads must be an association between two centromeres.

succeeding stages indicates that they are probably genuine examples of non-homologous association. In Text-fig. 5 the sex chromosomes can be distinguished as a small deeply stained mass close to, and attached to,

the nucleolus, and also attached by threads to two pairs of autosomes. Stage B is of very short duration, and represented only by a band one or two cells wide across the testis. The cell then increases rapidly in volume, and few intermediate cells are present before stage C is reached.

Stage C (Text-figs. 6, 7; Pl. 14, figs. 1–3). Nearly half the prophase cells are in this stage. At the beginning of it there is a very rapid increase in cell volume, and the autosomes become elongated and narrow, with chromomeres of varying size. The number of visible threads corresponds with the haploid number of autosomes (five), so the autosomes are still so closely paired that the pairs appear as single threads. The staining capacity of the autosomes varies, but generally it is proportional to their width, their largest chromomeres being deeply stained, and their small chromomeres and connecting threads paler. The nucleolus is a large stained blob, and embedded in it is a smaller and more deeply stained mass, the sex chromosomes.

Non-homologous association of chromosomes is a constant and characteristic feature of this stage, irrespective of fixation and staining technique. The sex chromosomes and the centromere and end regions of the autosomes have an attraction for each other, and in consequence the chromosome complement is joined up, end to end, centromere to end, and so on, in various ways which will be analysed in detail later in this paper.

Stage D (Text-figs. 8, 9; Pl. 14, fig. 4). Stage C passes gradually into stage D, which can be distinguished by the fact that the autosome threads, still so closely paired that no split is usually visible between them, are more deeply stained, thicker, and rather shorter.

Stage E (Text-figs. 10–13). Stage E can be distinguished from stage D by the disappearance of the nucleolus, leaving the sex chromosomes as a small dark blob in the nucleus. The paired autosome threads shorten somewhat, and gradually become more evenly thickened. Short portions can be seen to be double (Text-fig. 13). The amount of non-homologous association is reduced, but it is still appreciable. Stages D and E each occupy about a quarter of the whole prophase.

Stage F (Text-figs. 14–17; Pl. 14, fig. 5). Stage F is of very short duration, and has only been found in one of the animals examined. The autosome pairs contract in length, and become thicker and smooth in contour, so that they appear sausage-shaped. The sex chromosomes form a sphere, with diameter equal to the width of the autosomes. Presumably each stained body represents a pair of autosomes, each of which has already split into two chromatids, but there is no visible sign of any division into two, let alone four. At this stage the non-homologous associations are

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greatly reduced in number, and no non-homologous associations of centromeres were found. Stage F passes quickly into first metaphase, and both stages were found in the same follicle, in adjacent cells.
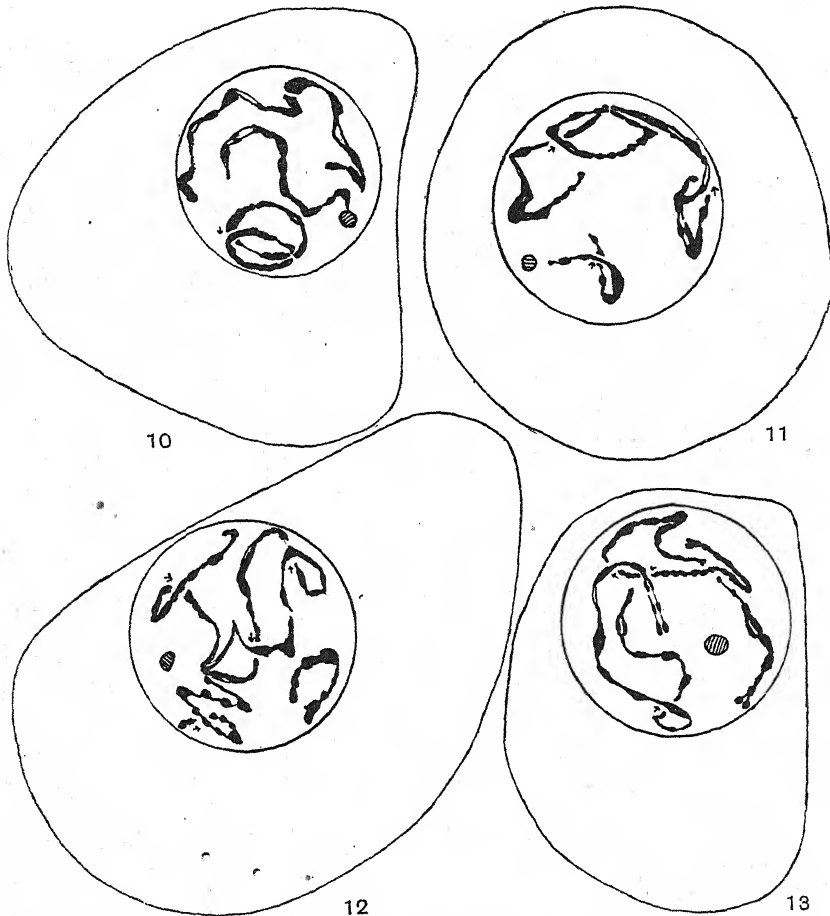
Text-figs. 10–13. Stage E. Text-fig. 10 shows a double loop formed by the association of both ends of two bivalents. In Text-fig. 11 there are two separate loops, each formed by the association of both ends of a bivalent. Text-fig. 12 shows an association of four ends of different bivalents. Text-fig. 13; a portion of the bivalent can clearly be seen to be double.

This description of the meiotic prophase in *Habropogon* resembles in many respects the descriptions of the same stages in other Asilids which have previously been given by Metz (1922) and Metz & Nonidez (1921, 1923, 1924). Stages A and B are closely comparable with their first two stages, which they find to be equivalent to those present in the late-

resting spermatogonia. It would seem that stages C and D are equivalent to pachytene in normal meiosis, and stage E, in which the threads have shortened, the nucleolus has disappeared, and most of the non-homologous centromere association has broken up, is equivalent to diplotene. Stage F is equivalent to diakinesis.

Metz & Nonidez have not reported any non-homologous association similar to that described in *Habropogon*, but they have reported that in
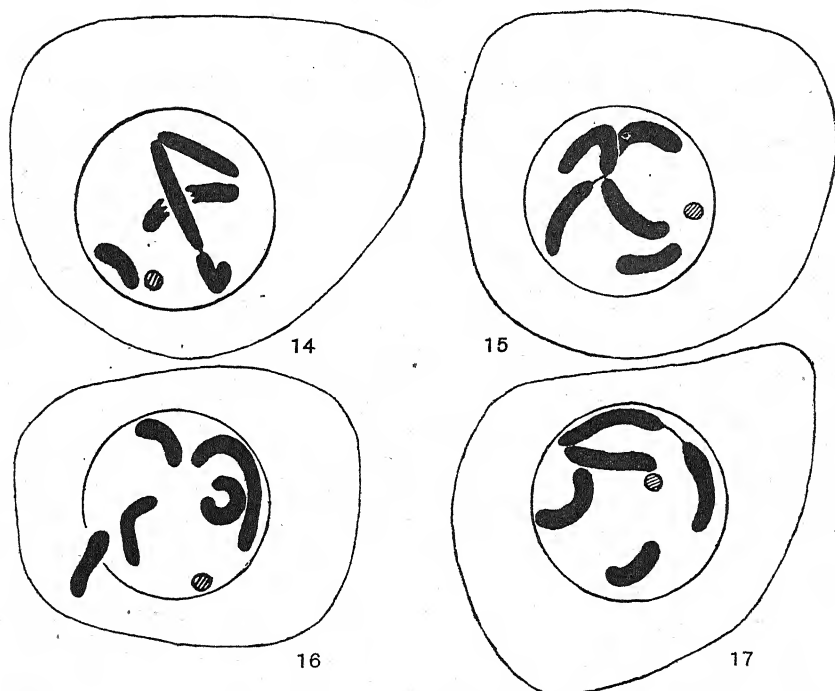
Text-figs. 14–17. Stage F. "Diakinesis." In Text-fig. 14 there is a chain of four bivalents, in Text-fig. 15 four ends form an association. Text-fig. 16 shows a chromosome in which the two ends have been joined to form a loop (cf. Text-figs. 10, 11), and in Text-fig. 17 three autosome pairs, together with the sex chromosomes, form a chain.

Asilus sericeus (1921) one end of each autosome is usually attached to the nucleolus sex-chromosome complex, and that there are usually some connecting threads between this structure and the autosomes in *A. notatus* (1923). In this latter species they report that at stage C the autosomes are so long and entangled that they cannot be traced individually, and in *Lasiopogon bivittatus* (1924) they report that the hyaline zones surrounding the chromosomes often join, so it is possible that some non-homologous associations were present but not analysable in this material.

5. MEIOTIC METAPHASE

Metaphase plates (Text-figs. 18, 19) show clearly the complement of five deeply stained autosomes, each with a submedian centromere, and in addition there is a short body, more palely stained and narrower in width than the autosomes, which is the sex-chromosome bivalent, which has now become undercondensed and understained.
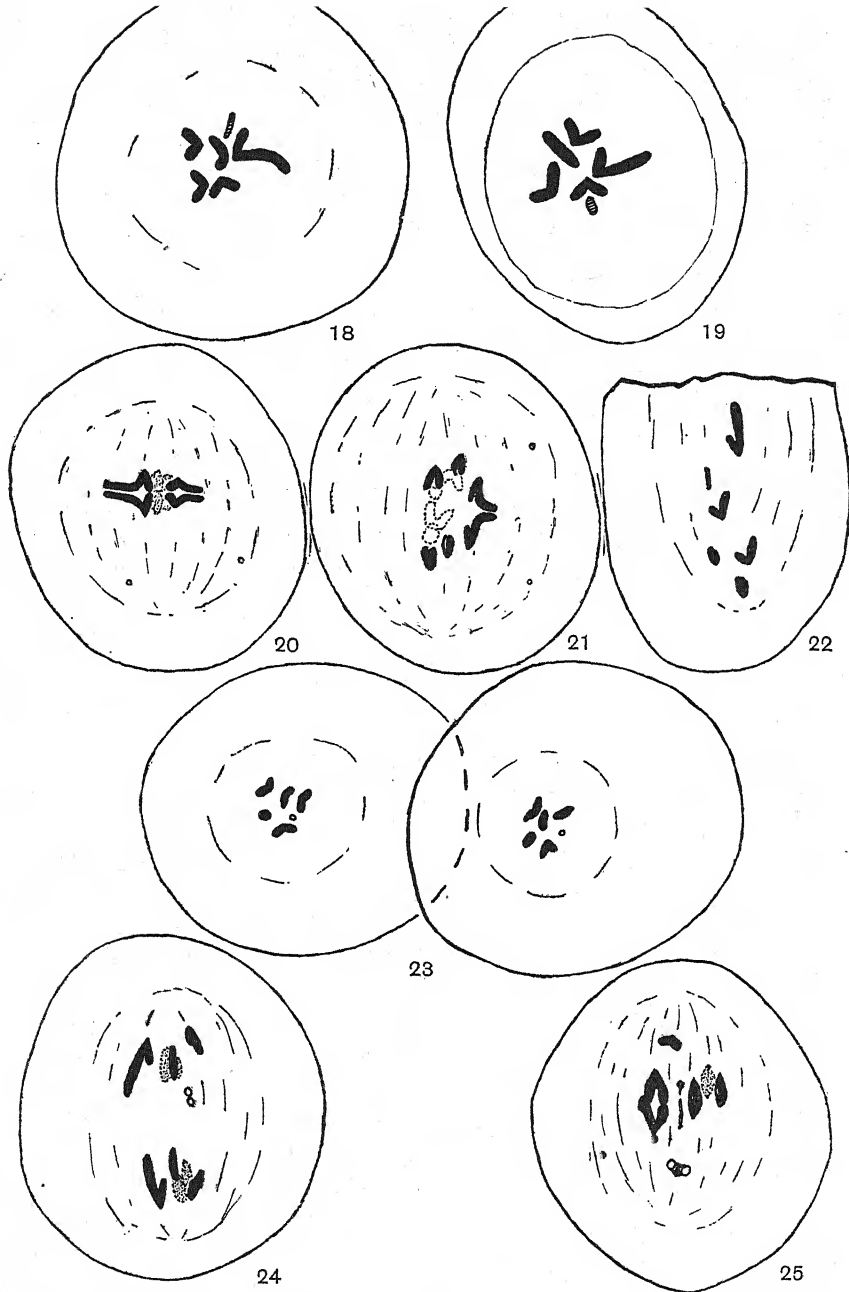
Side-views of first metaphase (Text-fig. 20; Pl. 14, fig. 7) and early anaphase (Text-fig. 21) show that the autosomes are held together in homologous pairs at metaphase by secondary pairing, and confirms the impression given by the prophases that chiasmata are formed. Thus Darlington's (1934) view that the mechanism of meiosis in Asilids is essentially the same as that in *Drosophila* is confirmed.

I have not been able to find certain evidence of the exact behaviour of the sex chromosomes at first metaphase, since I have not found any side view of metaphase in which they can be clearly seen, but a cell showing a pair of late first anaphases (Text-fig. 23) demonstrates that there is a sex chromosome at each pole, thus indicating that there is an XY mechanism, and the fact that the sex chromosome remains on the plate at metaphase is additional evidence in favour of this view. Darlington (1934) concluded that in *Drosophila* the sex chromosomes pair by reciprocal chiasmata, but it does not seem likely that this mechanism obtains in *Habropogon*, since the sex chromosomes form a rounded condensed mass, embedded in the nucleolus, throughout prophase, and their chances of forming chiasmata would seem to be remote.

In one cell (Text-fig. 24) the sex chromosomes appear to be lagging at anaphase, and in another (Text-fig. 25) two autosomes are off the plate, unpaired, at metaphase. They show position correlation, which indicates that they probably have been paired (cf. *Lilium* univalents, Ribbands, 1937). These cells indicate that meiosis by secondary pairing is occasionally imperfect, just as is the normal mechanism of meiosis by chiasma pairing.

6. ASSOCIATION OF NON-HOMOLOGOUS PARTS OF CHROMOSOMES

At all stages of meiotic prophase chromosome pairs were found associated with non-homologous pairs, either by their ends or by intercalary regions. At the later stages, C–F, and probably at stage B, these associations are characteristic and significant, since they occur irrespective of the fixative used, and their development and final dissolution can be traced in a logical manner through all the different stages of prophase which are examined.

Text-figs. 18, 19. First metaphase plates, sex chromosomes differentially condensed and
stained.

Text-fig. 20. First metaphase, side view, showing bivalents held together by secondary
pairing, without chiasmata.

Text-fig. 21. Similar attractions at early anaphase.

Text-fig. 22. Cut cell at anaphase, chromosomes disarranged, and differentially condensed
and stained sex chromosome clearly visible.

Text-fig. 23. Polar views of two halves of a dividing cell at first anaphase, showing sex
chromosome at both poles.

Text-fig. 24. First anaphase. Lagging pair of sex chromosomes.

Text-fig. 25. First metaphase. Two autosomes unpaired, off the plate. Unequal sex
chromosomes.

Most of the associations are terminal, but some are intercalary. No autosome pair has been found which possesses more than one point of intercalary association, and the positions of these associations correspond with the positions of the centromeres in the metaphase chromosomes. In the Hemipteran *Corixa punctata*, Slack (1938) showed that similar intercalary associations were always subterminal, and this fact could be correlated with the subterminal position of the centromere in this species. There is a tendency towards mutual attraction by centromeres at pachytene in the plant *Agapanthus* (Darlington, 1933) and in the grasshopper *Stauroderus* (Darlington, 1936), all the centromeres aggregate at one point in the spider *Schizocosa crassipes* at pachytene (Hard, 1939), and the centromere regions fuse to form a chromocentre in the salivary gland nuclei of most species of *Drosophila*. In view of these facts I conclude that the intercalary associations are centromere region associations, and I will henceforward refer to them as such.

7. FREQUENCY AND DISTRIBUTION OF NON-HOMOLOGOUS ASSOCIATION

Table 1 contains an analysis of the non-homologous associations during the last four stages of meiotic prophase. In all cases (except the simple configurations seen at stage F) each cell was drawn, and then analysed. In cases of doubt the end or centromere regions were counted as free. Note that throughout prophase homologues are so closely paired that they appear as single threads, hence what is referred to as a free end in this discussion is really the two closely paired ends of the homologues. The number of associated ends of the autosome pairs can be accurately determined by counting the number of free ends, but in cases where analysis was difficult the simplest configuration was assumed to be present, and in consequence the results probably slightly underestimate the amount of centromere region association, since it is possible to count an association of three threads as an association of three ends when it is really an association of one end with a centromere, and a junction of two ends elsewhere has been overlooked. Associations other than those of two ends cannot, of course, be overlooked. This source of error is probably the reason why the number of ends found associated in pairs at stages C and D is considerably less than at later stages, although the total number of associated ends is almost the same. In this case the number of ends found associated in threes is greater than expectation, and probably some of these are really junctions between ends and centromeres. The results given in brackets in Table 1 are based on the assumption that seven

Table 1. Frequency and distribution of non-homologous association

Note that n=5, and all the homologues are intimately paired. Hence each end or centromere referred to really consists of the pair of ends or centromeres of the two homologues.

The unbracketed figures represent the numbers actually counted, the bracketed figures represent the estimated frequencies after making allowance for a probable error in counting (see text).

Stage	No. of cells analysed	No. of free autosome pairs	No. of free autosome ends	Associations without sex chromosomes							Sex chromosome associations				Total no. of associated sex chromosomes	Total no. of associated centromeres	Total no. of associated ends	Proportion of associations of twos, threes, and fours
				End-to-end associations			Centromere associations				With one end	With two ends	With cen-tromere	Centro-mere and end				
				Two ends	Three ends	Four ends	With one end	With two ends	With three ends	With cen-tromere								
C	10	4	39	8 (15)	10 (3)	1	7 (14)	1	—	1	1	—	1	1	3	12 (19)	61	58%—39%—3% (77%—19%—4%)
Do. per cell	—	0·4	3·9	0·8 (1·5)	1 (0·3)	0·1	0·7 (1·4)	0·1	—	0·1	0·1	—	0·1	0·1	0·3	1·2 (1·9)	6·1	—
D	10	9	40	12 (15)	6 (3)	1	2 (5)	2	1	1	1	2	—	1	3	8 (11)	60	53%—39%—8% (68%—26%—6%)
Do. per cell	—	0·9	4·0	1·2 (1·5)	0·6 (0·3)	0·1	0·2 (0·5)	0·2	0·1	0·1	—	0·2	—	0·1	0·3	0·8 (1·1)	6·0	—
E	20	17	87	32	6	3	7	—	1	—	5	2	—	—	7	8	113	79%—14%—7%
Do. per cell	—	0·85	4·3	1·6	0·3	0·15	0·3	—	0·05	—	0·25	0·1	—	—	0·35	0·4	5·7	—
F	28	75	198	31	1	2	—	—	—	—	9	—	—	—	9	—	82	94%—2%—4%
Do. per cell	—	2·7	7·1	1·1	0·03	0·07	—	—	—	—	0·32	—	—	—	0·32	—	2·9	—

centromere-to-end junctions have been overlooked at stage C, and three at stage D. It will be seen that the numbers and proportions of end-to-end associations when compared with later stages then accord closely with expectation.

From the data given in Table 1, thus corrected, it is found that

(1) At stage C (= early pachytene) 61 % of the ends and 38 % of the centromere regions of the autosome pairs, and 30 % of the sex chromosomes, take part in non-homologous association. There is an average of 3·8 non-homologous associations per cell.

(2) At stage C 77 % of the associations involve two points, 19 % involve three points, and 4 % involve four points.

(3) At stage D (= late pachytene) the only significant change is a decrease in the amount of centromere region association from 38 to 22 %. Correlated with this the average number of non-homologous associations has dropped to 3·1 per cell.

(4) At stage E (= diplotene) the proportion of associated centromere regions has fallen to 8 % and the average number of non-homologous associations to 2·8 per cell. There has been little or no decrease in the number of end-to-end associations.

(5) At stage F (= diakinesis) no centromere region associations were observed. The number of associated autosome ends has dropped from 57 to 29 %, but the proportion of associated sex chromosomes shows no change. There is an average of 1·5 associations per cell.

(6) The cells counted at stage F were all in one follicle, cut horizontally. Adjoining them were at one end stage E cells, at the other metaphase cells. They spread through five sections. Counts of each section showed 15 out of 40, 9 out of 20, 34 out of 110, 20 out of 90, and 4 out of 20 associated ends respectively, when the sections were examined in order from stage E to metaphase. The χ^2 can be calculated after grouping together the first two and the last two of these readings. $\chi^2 = 5\cdot854$, and since there are then two degrees of freedom, the probability that these end associations were distributed randomly is about 1 in 18. It is therefore highly probable that the non-homologous association shows a progressive decrease throughout stage F.

(7) The relative proportions of the associations of twos, threes, and fours at different stages indicate that associations of three are more unstable than associations of either two or four, and break up first. If further work shows that this is in fact the case, it will be of some theoretical interest, and may imply that I have tended towards over-correction of the stage C data, and slight over-estimation of the amount of centromere

region association at this stage; but the uncorrected data yield exactly the same general conclusions.

(8) If the chance of association of centromere regions and ends is random, and the frequency of association of both ends and centromere regions is known, the percentage of free autosomes can be calculated. At stages C, D, E and F the expected percentages of free autosomes, calculated from the data given in Table 1, are 10·3, 13, 17, and 51 respectively, and since Table 1 shows that 8, 18, 17 and 54 % respectively were found, the distribution of the free autosomes shows that the association could have been random. Unfortunately this does not prove that the association was random, since this index is not a sensitive one, and a fairly considerable departure from randomness might be made in certain directions without marked effect upon the expected number of free autosomes.

Summarizing the more important of these results, it appears that at early prophase about 60 % of the ends and 40 % of the centromere regions of the autosome pairs, together with 30 % of the sex chromosomes, take part in non-homologous associations, the average frequency of associations being 3·8 per cell. About 75 % of these associations are of two points only, and none of more than four have been found. The centromere region associations break up progressively during prophase, and have all gone before stage F, equivalent to diakinesis, is reached. The autosome end associations break up rapidly during stage F, but the sex chromosome associations may be more persistent. Possibly these latter survive to the onset of metaphase. The data will fit the assumption that association is random, but randomness cannot be proved.

8. Comparison of frequency of non-homologous association in *Habropogon* and *Corixa*

Habropogon appendiculatus has two advantages not possessed by *Corixa punctata*—a smaller chromosome complement and submedian instead of subterminal centromeres—and in consequence it has been possible to make a fuller statistical analysis than that which was attempted by Slack (1938). It has been found more convenient to present the data for *Habropogon* in a different form, but certain comparisons may be made, and these are summarized in Table 2.

It may be noted that in both species an end stands considerably more chance of entering an association than a centromere region.

In *Habropogon* the centromere region associations break up gradually

through prophase, but the end associations remain constant, and only break up at a stage equivalent to diakinesis. In *Corixa* the end associations go earlier and nearly half the bivalents are free by late diplotene. This can probably be associated with the difference in contraction of the autosomes. Whereas in *Corixa* there is a contraction at metaphase to one-seventh of the early diplotene length, and this contraction has developed considerably by late diplotene, in *Habropogon* very little contraction occurs before "diakinesis", and the total contraction is only to one-third of the early prophase length.

Table 2. *Comparison of non-homologous association in* Corixa punctata *and* Habropogon appendiculatus

| | Pachytene or stage C | | | | | Late diplotene or stage E. | Diakinesis or stage F. |
	Proportion of centromere association to total association %	Ends in associations %	Centromeres in associations %	Free sexchromosome pairs %	Free autosome pairs %	Free autosome pairs %	Free autosome pairs %
Corixa	13[1]	72[2]	22[3]	73[4]	4[4]	41[4]	70[4]
Habropogon[5]	24	61	38	70	8	17	54

(1) Slack was unable to analyse the associations of more than two chromosome pairs, but he found 46 associations containing two pairs only in 23 pachytene nuclei. From his data one can calculate that 12 of these 46 associations were centromere to end associations, hence (1).
(2) Slack's first table shows that there were 108 free ends in 23 pachytene nuclei. 70% of the sex chromosomes are free, and therefore, since there are 11 autosome pairs, there are 69 free autosome ends out of 253, hence 72% of the ends are in associations.
(3) Calculated from (1) and (2).
(4) Calculated from Slack's second table.
(5) Calculated from Table 1.

As in *Habropogon*, when the percentage of ends in associations and the percentage of centromere regions in associations is known, the expected percentage of free autosomes if association is random can be calculated. At pachytene the calculated percentage of free autosomes in *Corixa* is 6, and the counted percentage is 4, so the associations could be random. But, as in *Habropogon*, it must be stressed that this calculation does not prove random distribution, it only shows that the data are compatible with random distribution.

The percentage of free ends and free centromeres is a much better guide for quantitative studies than the percentage of free autosomes, since at high frequencies of association very few autosomes are free, and, moreover, significant data can be obtained from a much smaller number of nuclei.

9. The relation between the nucleolus and the
sex chromosomes

In *Habropogon appendiculatus* the sex chromosomes are precociously condensed, and closely attached to the nucleolus throughout meiotic prophase until the nucleolar material at stage E ceases to stain and disappears. In three other Asilids, *Asilus sericeus* (Metz & Nonidez, 1924), *A. notatus* (Metz & Nonidez, 1923) and *Lasiopogon bivittatus* (Metz & Nonidez, 1924) the sex chromosomes are similarly related to the nucleolus, and a similar relationship holds in some Hemiptera, e.g. *Corixa punctata* (Slack, 1938); in some Orthoptera, e.g. *Stenobothrus* (Davis, 1908), *Chorthippus* and *Stauroderus* (Darlington, 1936); in some Lepidoptera, e.g. *Philosamia* (Dederer, 1907) and in the Chilopod *Scolopendra* (Blackman, 1905).

In related species, when the nucleolus is attached to autosomes, there is a normal spherical nucleolus attached to a constriction in the autosome, e.g. in the Asilid *Dasyllis grossa* (Metz, 1922), but in the instances of nucleolar attachment to the sex chromosome the nucleolus usually enters into a closer relationship, no attachment constriction is visible and the nucleolar material usually partly or completely covers the sex chromosomes.

Two alternative hypotheses would account for this difference in behaviour:

(1) That in the cases where the nucleolus envelops the sex chromosomes there are no specific nucleolar attachments, and in consequence the nucleolar material tends to become aggregated round the chromatin, as in the abnormal maize described by McClintock (1934), but that it has a special attraction for heterochromatin and therefore collects round the sex chromosomes.

(2) There are specific nucleolar attachments in the sex chromosomes, and the difference in nucleolar behaviour is ascribed to the special properties of the sex chromosomes.

The second explanation must be the correct one, because:

(*a*) If the association between nucleolus and sex chromosomes were due only to a special attraction between nucleolar material and heterochromatin the very large blobs of heterochromatin at many chromosome ends in *Corixa punctata* (Slack, 1938) would also be covered in nucleolar material, but they are not, and hence the embedded sex chromosomes, restricted by nucleolar material, enter into non-homologous association

much less frequently than the heterochromatic masses at the autosome ends.

(*b*) Even where the sex chromosomes are small and appear to be completely embedded in nucleolar material, as in *Habropogon*, the nucleolar material does not form an even coat around the sex chromosome, but is mainly aggregated to one side of the sex chromosome (Text-figs. 5–9; Pl. 14, fig. 3). This indicates a definite attraction for some specific part of the chromosome.

(*c*) In some related species the nucleolar material remains unattached and does not enter into relations with any of the chromosomes, and tends in fact to be repelled by all of them, including the precociously condensed sex chromosomes, e.g. in the Orthopteran *Arphia tenebrosa* (Davis, 1908) and the Hemipteran *Aphelocheirus aestivalis* var. *Montandoni* (Slack, unpublished).

Hence it must be inferred that there are specific nucleolar attachments in those sex chromosomes which appear to be wholly or partly embedded in nucleolar material, and that the different nucleolar behaviour is due to a special property of the sex chromosomes, and presumably this special property is the presence of heterochromatin.

McClintock (1934) showed that in maize the nucleoli originate from an organized body in the chromosome, and the stalk or secondary constriction was produced as the result of growth of the nucleolus, and the constriction was proportional to the size of the nucleolus, and that when no nucleolus was formed at a region containing the nucleolar organizer no secondary constriction was formed either. This latter observation was also made by Navashin (1934), working on *Crepis*. Resende's work (1939) appears to conflict with this view, yet it still seems the most reasonable one, as Melland (1939) points out. Now under many circumstances there is a strong repulsion between nucleolar material and euchromatin, and this repulsion is seen, for instance, in prophase of *Asilus notatus* (Metz & Nonidez, 1923), and in similar stages of another Asilid, *Machimus caliginosus* (Ribbands, unpublished). In *M. caliginosus* this repulsion may cause the nucleolus to be pushed to one side of the nucleus and to deform the nuclear membrane, and lie in a pocket in it. It may be supposed that this repulsion, which in *Asilus notatus* and *Machimus caliginosus* causes any attachments between the autosomes and the nucleolus to be drawn out into threads, also causes the uncoiling of chromosomes in the region of nucleolar attachment in other cases, and is responsible for the secondary constrictions which are observed. Now heterochromatin differs from euchromatin particularly in having much less of the materials, or pro-

perties, responsible for mutual repulsion between euchromatin and nucleolar material. This fact would account for the behaviour of the nucleolar material towards the condensed sex chromosomes in the instances noted above.

10. NON-HOMOLOGOUS ASSOCIATION AND NUCLEOLAR MATERIAL

McClintock (1934), during her studies on *Zea*, found that changes in the chromosomes leading to genetic unbalance sometimes prevent the usual organization of the nucleoli. The nucleoli then appear as droplets generally distributed over the chromosomes, and these droplets may fuse so that the chromosomes appear to enter into a form of non-homologous association, as an entangled and irregular mass. Darlington (1936) considered it probable that the deposition of nucleolar material on the precocious sex chromosomes of *Chorthippus* and *Stauroderus* would account for the attraction which existed between these sex chromosomes and the ends of the paired autosomes. He suggested that small quantities of nucleolar material were deposited at the autosome ends, and that this would account for their attraction to the sex chromosomes and for the mutual attractions which they exhibited at the polarization centres.

I believe that many of the phenomena described by Darlington are essentially similar to the non-homologous associations in *Corixa punctata* and *Habropogon*, and that they cannot be attributed to attractions between nucleolar material, because:

(1) There is usually a repulsion between chromatin and nucleolar material, as I have illustrated in the preceding section, and this repulsion would tend both to prevent the deposition of nucleolar material as postulated and to prevent it from performing the functions assigned to it when so deposited.

(2) Slack (1938) showed that in *Corixa* the swollen ends stained by Feulgen's technique in a similar manner to the rest of the chromosomes, whereas nucleoli do not usually stain by this process.

(3) In *Corixa* and in *Habropogon* the nucleolar material seems to disintegrate after mid-diplotene or stage D, but the non-homologous associations persist to diakinesis. In *Chorthippus*, too, drawings (Darlington, 1936, text-figs. 20c, g, h) indicate the persistence of non-homologous association after the disappearance of the nucleolus.

(4) The clearest instance of ends of non-homologous chromosomes being brought close together by nucleolar material is provided by *Fritillaria*. In *F. elwesii*, for example, Darlington (1935) shows that four pairs of chromosomes have nucleoli attached very close to their proximal

ends at early diplotene. By late diplotene the nucleoli have fused, so the proximal ends of these chromosomes have been brought close together. During diakinesis the fused nucleolus disintegrates, and no evidence of any non-homologous association by the ends bearing nucleolar attachments is reported.

(5) Conclusive proof that nucleolar material plays no part in forming the non-homologous associations of *Corixa* and *Habropogon* is given in Table 2, where it is shown that the sex chromosomes, which are covered in a large coating of nucleolar material, show only 30 % of non-homologous association, whereas 90 % of the autosome pairs enter non-homologous associations in both cases. Since the alternative explanations of non-homologous associations either postulate attractions between heterochromatic regions, and the sex chromosomes are heterochromatic, or attractions between centromeres and ends, which are present in equal numbers in sex chromosomes and autosomes, it would seem likely that the nucleolar material surrounding the sex chromosomes in these cells is a hindrance to non-homologous association.

11. Functions of the nuclear membrane

In *Habropogon* the chromosomes are not dispersed at random in the nucleus. From stage C onwards they tend to lie round the periphery of the nucleus, and the centres of non-homologous association are usually very close to the nuclear membrane. In this respect they resemble the condition found in *Corixa punctata*, in which Slack (1938) reports that the largest centres show the greatest regularity in taking up a position near to the nuclear membrane. In Orthoptera Davis (1908) pointed out that the centres of polarization were close to the nuclear membrane, and numerous subsequent workers have also found this, and in this instance it is especially interesting because chromosomes are distributed through all regions of the nucleus. In spiders, Hard (1939) describes an interesting example in which all the centromeres become attached to one point on the nuclear membrane, so that all the chromosomes become polarized about this point.

Hence it may be concluded that non-homologous associations between ends and centromere regions occur in the vicinity of the nuclear membrane. Slack (1938) suggests that the surface charge which the chromosomes seem to carry and which would cause their mutual repulsion (cf. Lillie, 1905; Kuwada, 1929) is greatest in the large heterochromatic masses at the non-homologous centres in *Corixa*, so causing greater repulsion towards the periphery, but such an explanation would not

account for the peripheral position in the other instances mentioned, where large heterochromatic masses are not usually present. It would seem that the regions which enter into non-homologous association do possess some property which causes them either to be attracted towards the nuclear membrane, or to be repelled from the centre of the nucleus, and that this property bears no relation to the size of their surface area. It might be suggested that they are attracted towards the centrosomes outside the nucleus, but in *Habropogon* and *Corixa* there are numerous associations, and there cannot be more than two centrosomes, and other evidence (see below) causes me to postulate a lack of centrosome attraction in these cases.

Another probable function of the nuclear membrane is an effect on precocious condensation. In many organisms parts of some autosome pairs thicken precociously and stain more deeply than other parts, but there is no necessary constancy about this precocity, e.g. in eight drawings of prophase cells of *Hyacinthus orientalis* (Upcott, 1938, text-figs. 3–10), where one member of the chromosome complement can be recognized by the presence of a nucleolus, the end of the nucleolar chromosome distal to the nucleolus in two cases shows precocious condensation and in three cases shows subnormal condensation. Similar irregular precocious condensation occurs in *Habropogon*, and it was noted that it was visible most frequently (16 times out of 20) in the short arm of the short L-shaped chromosome. It is of course this arm which will usually lie nearest to the nuclear membrane, since its centromere and end regions are both frequently in associations close to it. Now in many organisms, e.g. *Fritillaria* (Darlington, 1935), precocious condensation is associated with earlier pairing, and while with irregular arrangement it is difficult to trace the course of pairing, it has frequently been observed that in cases of bouquet arrangement pairing begins at the ends lying towards the surface of the nucleus, whether the centromere is situated there or not (Wenrich, 1916; Gelei, 1921; Belar, 1928; Darlington, 1937, p. 91). Furthermore, it has frequently been observed that pairing begins near the centromere, and the evidence reviewed above indicates that there is often a special force acting on the centromere region and tending to bring it close to the nuclear membrane. All these items, small in themselves, lead towards the tentative conclusion that proximity to the nuclear membrane tends to lead to precocious development. The fact that this development can be very variable, e.g. in *Hyacinthus*, indicates that it is unlikely that precocious development causes the proximity to the nuclear membrane. Recent work indicates a possible explanation of this function of the

nuclear membrane, since Caspersson & Schultz (1939) believe that synthesis of nucleotides, precursors of nucleic acids, occurs at the nuclear membrane.

These probable functions of the nuclear membrane add complications to considerations of non-homologous association, since there are many instances in which they have been attributed to heterochromatin, the only evidence for which is the precocious condensation of the associated regions close to the nuclear membrane.

12. THE MECHANISMS OF NON-HOMOLOGOUS ASSOCIATION IN OTHER ANIMALS AND PLANTS

For some time work upon non-homologous associations of chromosomes has been dominated by the hypothesis of Heitz (1929) that regions of chromosomes which showed differential response to staining were inert, and by the demonstration by Prokofieva (1935) that the chromocentre of salivary gland nuclei of *Drosophila* was formed by the non-homologous association of the heterochromatic centromere regions. Genetic evidence (e.g. Fujii, 1939) has shown that the heterochromatic regions in these nuclei correspond very closely in position with the genetically inert regions. That these heterochromatic regions do possess the property of non-specific attraction, and that the chromocentre is not produced merely by the fusion of centromeres and the materials adjoining them, is proved by the work of Schultz (1936) who showed that when, in "variegated" races of *Drosophila*, portions of the heterochromatin are transferred to inter-calary regions, these portions still become attached to the chromocentre, and chromosome loops are formed.

Hence the very reasonable hypothesis that heterochromatic regions were inert, and that their attraction for similar regions was really an attraction between similar (inert) genes, as Prokofieva maintained. Bauer (1936a), however, disputed this on the ground that there were at least two different kinds of heterochromatin, "loose" and "compact", and there-fore that the attraction was an attraction between non-homologous regions.

Unfortunately, these quite simple hypotheses are complicated by other factors. First, Fujii (1939) has shown that in *Drosophila virilis* the proximal half of the third chromosome is genetically inert, and this region contains no heterochromatin (Heitz, 1934), while on the other hand some heterochromatic regions are not inert, e.g. the *X*-chromosome of *Sphaerocarpus donnellii* is heterochromatic, but produce very frequent mutations, with considerable structural effects (Knapp, 1935a, b).

Hence there seems to be no absolute correlation between inertness and heterochromatin. The relation is, however, a usual one, and this is confirmed by recent work on *Drosophila*, in which it has been demonstrated that euchromatin can be converted into heterochromatin if a small euchromatic region is translocated into a large heterochromatic region, and that at the same time the genes in the translocated region become recessive instead of dominant, and may become inert (Schultz, 1939).

In the salivary gland nuclei of *Chironomus* (Bauer, 1935; Poulson & Metz, 1938), *Drosophila* (Bauer, 1936 b) and *Sciara* (Poulson & Metz, 1938), where heterochromatic regions can easily be detected, these workers find that, in addition to the attractions between non-homologous heterochromatic regions, there is attraction between chromosome ends where no heterochromatin is present. The recent work of Hard (1939) on the spider, *Schizocosa crassipes*, shows conclusively mutual attraction between centromeres, which become attached to the nuclear membrane at pachytene, and this attraction, too, cannot be due to heterochromatic regions, since the rest of the chromosomes are condensed and not mutually attracted, and the centromere attraction is at the least condensed portion.

Hence examples of non-homologous association elsewhere should be analysed, if possible, in order to determine whether they are in fact due to heterochromatin, or to mutual attractions between centromeres or ends, or to yet another mechanism.

In *Drosophila*, thanks to the genetic studies which enable the presence of genetically inert material to be detected, and to the special form of the salivary gland chromosomes which show clearly the position of the heterochromatin, precise results are obtainable, but elsewhere neither genetic nor cytological technique is a thoroughly reliable guide to the presence of heterochromatin. It should be noted that even in *Drosophila* opinions differ concerning the distribution of heterochromatin, since Bauer (1936 b) considers that there is no heterochromatin at the ends of the autosomes which help to compose the chromocentre in *D. funebris*, while Emmens (1937) considers that there is.

As one example of the way in which theoretical bricks can be made without much factual straw may be mentioned the announcement of Kostoff & Arutinian (1938) that they had demonstrated heterochromatic regions, variable in length, in the anaphase chromosomes of *Crepis capillaris* by a destaining technique. From this they inferred that non-homologous association occurred at these regions. However, Richardson had previously (1935) recorded extensive observations on *C. capillaris*, and found that pachytene was quite normal, with no sign of non-homo-

28–2

logous association, and no precocious condensation of any chromosome regions, so it would seem that the technique of Kostoff has not yet been proved to be a good guide to the presence of heterochromatin, and hence his demonstration of heterochromatin at the ends of *Triticum monococcum* (Kostoff, 1938) cannot be considered conclusive, and it is not proved that the end-to-end associations in this species are due to the presence of heterochromatin. In this connexion it may be noted that the demonstration of heterochromatin in *Paris* metaphase chromosomes by Darlington & La Cour (1938), shows heterochromatin understained and differentially condensed, just as it is normally elsewhere when differentiated at this stage (e.g. in the sex chromosomes of *Habropogon*) and not overstained, as in Kostoff's examples.

On the other hand, the work of McClintock (1930) and Longley (1937, 1939) has demonstrated the presence of knobs, constant in position, on the prophase of chromosomes of *Zea*, *Euchlaena* and their hybrids. It seems fairly certain that these structures are formed of heterochromatin, and they have been proved to enter into frequent mutual non-homologous association. In *Zea* the knobs form at different points along the length of the chromosome, but in *Euchlaena* nearly all are terminal, and most ends are knobbed, so the distribution of the heterochromatin is similar to that which would need to be postulated in *Habropogon* if these associations are due to heterochromatin.

In *Corixa punctata*, Slack (1938) has shown that large masses of heterochromatin are present at some of the ends which enter into non-homologous association, and he therefore suggests that heterochromatin is the most likely cause of these associations. He also points out that the numerous scattered associations found in *C. punctata*, and similar to those described in *Habropogon*, do not appear fundamentally different from the single or few large associations found in polarized cells at pachytene in Orthoptera. It is with these latter cells, examined extensively by many workers that I will now deal.

In Orthoptera the pachytene chromosomes usually traverse the nucleus in all directions, but in one, or two, or several places there are associations of ends or centromere regions of non-homologous pairs. Frequently all the ends are in these associations (e.g. *Dissosteira carolina*, Davis, 1908), but at other times only the proximal ends attach (e.g. *Mecostethus grossus*, Janssens, 1924), and in some cases some chromosomes are free (e.g. *Stenobothrus biguttulus*, Gérard, 1909). Precocious condensation has frequently been demonstrated at the ends which join to form these associations, either in the form of large masses of chromatin

(e.g. *Phrynotettix magnus*, Wenrich, 1916; *Stauroderus scalaris*, Corey, 1933; *Oedipoda miniata*, etc., Corey, 1938) or in the form of small deeply staining granules (e.g. *Hippiscus tuberculatus*, Davis, 1908; *Mecostethus grossus*, Janssens, 1924; *Ageneotettix deorum*, Corey, 1938), but it must not be overlooked that in other species with similar associations there is often no evidence of such precocious condensation (e.g. *Dissosteira carolina*, Davis, 1908, fig. 33; Corey (1933) says there is no proximal condensation in *Stauroderus bicolor* ends, in which Darlington (1936) reports association). Furthermore, in *Periplaneta americana* (Morse, 1909) the chromosomes are clearly shown to be proximally condensed but to have their distal ends, and not their proximal ends, polarized. This is also true of *P. australis* (Ribbands, unpublished). Hence it can only be concluded that the evidence concerning the presence of heterochromatin at the polarized ends and centromeres of Orthopteran chromosomes is contradictory, but that it does not warrant the conclusion that heterochromatin is necessarily concerned in these associations. Indeed, in view of the fact that these associations are usually at the nuclear membrane, some of the reported instances of precocious condensation may be due to this factor, and not to heterochromatin.

Two further interesting points emerge from a study of these cells in Orthoptera, both concerning the sex chromosomes. These chromosomes are heterochromatic, and presumably if the polarization were due to attractions between heterochromatin they would be expected to play a prominent part, and the whole chromosome, and not merely a part of it, would take part in the association. In practice this rarely occurs, and the fact that the nucleolus often surrounds part of the sex chromosome (e.g. *Stenobothrus curtipennis*, Davis, 1908; *Chorthippus parallelus*, Darlington, 1936) will account for this in many cases, in view of its restrictive effect on these associations, mentioned above. However, in both *Dissosteira carolina* and *Arphia tenebrosa*, Davis (1908) has shown that the nucleolus is separate from the sex chromosomes, and yet in both these species the markedly heterochromatic sex chromosomes are not the centre of non-homologous association, as would be expected if the associations were due to heterochromatin, but are usually attached non-homologously only by an end which is often drawn out towards the point of association.

The work of Darlington (1936) is also suggestive. I notice that the associations described by him in *Stauroderus bicolor* can be divided into three types: (*a*) the association of parts of the precociously condensed autosomes P, S, and M7 with the precocious sex chromosomes; (*b*) the polarization of many of the ends of the autosomes at one or more centres,

(*c*) in one instance there is evidence of a mutual attraction between three centromeres, and in another of mutual attraction between a centromere and four ends. The first of these types of association, between precociously condensed chromosomes, seems to be a genuine example of mutual attraction between heterochromatic regions, and it should be noted that this type of association differs from the other two types, and from the *Habropogon* associations, in that it is often intercalary, and these intercalary junctions are *not* situated at centromeres (Darlington, 1936, text-figs. 16, 20 *d, f, g, j, m*), and there is visible heterochromatin. The other two types of association found occur only at ends and centromeres, they are apparently not associated with heterochromatin (Corey, 1933), and they would seem to be essentially similar to those of *Habropogon*.

Summarizing the conclusions to be drawn from the above data, the non-homologous associations between chromosomes may be classified as shown in Table 3.

Table 3. *Analysis of mechanism of non-homologous association*

Heterochromatin attractions	Heterochromatin always present	No visible heterochromatin	End or centromere attraction, no heterochromatin
1. Chromocentre of *Drosophila*	1. Some Orthopteran polarization, e.g. *Stauroderus scalaris*	1. Some Orthopteran polarization, e.g. *Dissosteira carolina, Periplaneta americana*	1. Distal ends of Dipteran salivary gland chromosomes
2. Knob associations in *Zea* and *Euchlaena*	2. Many ends of *Corixa punctata*	2. *Habropogon appendiculatus* associations	2. Centromere polarization in spider *Schizocosa crassipes*
3. Precocious chromosomes in *Stauroderus*			

We thus see that there are definite instances in which heterochromatin attractions give rise to non-homologous association, and other definite instances in which mutual attractions between chromosome ends or centromeres must be postulated. Intermediately situated are a large number of examples in which heterochromatin may or may not be visible, and in which it is difficult or impossible to prove which is the effective mechanism, or whether both are effective together.

There is no clear evidence to indicate whether there may be attraction between heterochromatin and non-heterochromatic ends or centromeres, but this possibility must be remembered. There is some evidence of a difference in behaviour between the two types of association. First, heterochromatic attractions may be stronger (cf. *Drosophila* proximal and distal end attractions), and secondly, they may be more persistent, as shown in *Stauroderus bicolor*, where drawings (Darlington, 1936) indicate

that the heterochromatic precocious chromosomes association may persist to diplotene, but the associations between apparently non-heterochromatic ends do not. This might, however, be due to the premature shortening of the former chromosomes at pachytene, which would result in less strain during diplotene shortening. These points need further inquiry.

13. The mechanism of non-homologous association in *Habropogon*

The evidence reviewed above indicates that non-homologous association in *Habropogon* might be due either to the presence of heterochromatin or to special attractions of ends and centromeres, or to both of these mechanisms. It is unfortunately not possible to determine with certainty which is the operative cause, but the following evidence points towards the conclusion that it is not heterochromatin:

(1) In some of the non-homologous associations in *Habropogon* the associated portions, far from being precociously condensed, are palely stained and finely drawn-out threads, with no sign of even a deeply stained terminal granule (Text-fig. 7).

(2) In no case is there conclusive positive evidence of the presence of heterochromatin.

(3) If heterochromatin were the determining factor the sex chromosomes, which are always precociously condensed at prophase, should be associated much more frequently than any other regions, but they show only 30% of association at pachytene, compared with 90% shown by the paired autosomes. It is doubtful if this difference could all be attributed to repulsions due to the attached nucleolus if heterochromatin were the attracting force.

(4) If association were due to heterochromatin, one would expect that (*a*) heterochromatin would not necessarily be present at all ends (Text-fig. 6 shows all ends except one in association); (*b*) although there are theoretical reasons why it should not occur elsewhere as well—yet no instances of intercalary non-centromere association were found, and small portions of chromosomes only were involved in associations, not long regions as might be expected when the large areas of heterochromatin present in *Drosophila* are remembered.

(5) In heterochromatin associations, the frequency of association might be expected to be proportional to the quantity present, and then some regions would enter associations much more frequently than others. The prophase chromosomes of *Habropogon* have no distinguishing markers

by which they can be readily identified, so this possibility cannot be conclusively tested, but it may be noted that the data concerning expected and known frequency of chromosome association in *Habropogon*, analysed previously, are compatible with a random distribution of frequency of association, which would be expected if special end and centromere attractions were the cause of the associations.

Thus I tentatively conclude, for the above reasons, that the non-homologous associations in *Habropogon* are due to special mutual attractions between centromeres and ends. It may be noted that all the above arguments, except the first two, apply also to the associations in *Corixa punctata* (Slack, 1938).

It is clear that direct observation of these small chromosomes is not sufficient to provide evidence of the presence or absence of heterochromatin. In my opinion it is only by extensive statistical analysis of very favourable material that random distribution of the associations could be proved or disproved. Proof of randomness would discredit the heterochromatin hypothesis in such a case, proof of non-randomness would indicate that heterochromatin attractions were present, but would not eliminate the possibility that other attractions were also in action.

14. ATTRACTIONS BETWEEN CENTROSOMES, CENTROMERES AND CHROMOSOME ENDS

Prophase polarization of chromosomes has so far been discussed as if it were essentially similar to the non-homologous associations found in *Habropogon* and *Corixa punctata*, and there seems to be only one significant difference between the two types of association, namely, that in the former case only one or two large associations are formed, and in the latter there are a number of associations, and small associations (twos) predominate.

Now the attractions causing non-homologous association are not ones which can be satisfied by association, as in the case of the gene for gene attraction, otherwise associations of two only would be formed. If the attraction were greater when a greater number of centromeres or ends were present large accumulations of such regions in one spot might be expected to result, and this could account for the conditions found in polarized nuclei. Yet another factor also needs to be taken into account, since when the position of the centrosomes has been observed in polarized nuclei they have been found to be in the cytoplasm near to the part of the nuclear membrane at which the ends or centromeres are polarized (*Periplaneta americana*, Morse, 1909; *Mecostethus grossus*, Janssens, 1924;

Dendrocoelum lacteum, Gelei, 1921). Hence it seems probable that the centromeres and ends which are polarized are attracted towards the centrosomes, and that this attraction accounts for the large associations found in polarized nuclei. Probably some different condition of the cytoplasm or centrosomes is responsible for the absence of centrosome attraction in *Habropogon* and *Corixa punctata*.

Janssens himself (1924) pointed out that sometimes there are two or more centres of association in polarized nuclei, and that there cannot be more than two centrosomes. He only found that the centrosomes were close to the associations while undivided, so that they would only account for one of the centres, but Morse (1909) found that after centrosome division both centrosomes acted as foci of association. The fact that there are sometimes three or more centres of association does not mean that the centrosomes play no part in the association. They are concerned with the largest centres, and just as in *Habropogon* and *Corixa*, although all the ends and centromere regions probably possess the property of mutual attraction some of them are left out of association in many cells, so in polarized nuclei we may suppose that in some instances the attractions are not sufficiently strong to bring all the ends or centromeres together, and secondary centres are formed.

Just as the power of centrosome attraction seems to vary from nil in *Habropogon* to a considerable power in *Chorthippus* and the power to attract all ends in *Periplaneta*, so the power of centromere region attraction varies. In *Habropogon* and *Corixa punctata* (where the centrosome attraction is least) centromere attraction is less powerful than end attraction, while in most Orthoptera the centromere attraction is more powerful than end attraction, and all proximal regions, whether end or intercalary, are polarized, while distal end regions are often not. *Periplaneta* is exceptional, since here apparently ends associate, centrosome attraction is marked and centromere attraction is absent.

It should be noted that the relationship between centrosomes and centromeres provides further indirect evidence that polarization is not controlled by heterochromatin. As Darlington (1937, p. 539) has pointed out, "the centromeres resemble the centrosomes in their permanence, in their dimensions (so far as these are constantly recognizable), in their cyclical staining properties, and in their correlated division and repulsion cycles. They differ from the centrosomes, however, in the timing of their division and in its effects." Hence we might expect a mutual attraction between centrosomes and centromeres, but there are no grounds for expecting an attraction between centrosomes and heterochromatin.

Thus the data reviewed indicate that there are many grounds for postulating attractions between centrosomes, centromeres and ends, but that these three things have different intensities of attraction, since in *Habropogon* and *Corixa* (*a*) the centrosomes play no part, (*b*) the centromeres at early prophase show less non-homologous association than the ends, and (*c*) the end associations persist longer than the centromere associations.

While these theories concerning the cause of non-homologous association postulate new properties for both centrosomes and centromeres, these properties are consistent with the already known properties of these and similar materials. In the case of the postulated attractions of chromosome ends, however, this cannot be said. Yet for a long time there has been evidence that chromosome ends possess special properties of their own, as is indicated by the fact of terminal chiasmata, and also by the evidence of X-ray breakage which indicates that breakage ends do not unite with free ends (Stadler, 1932; Helwig, 1938). It is interesting that Helwig (1933), has, however, reported that chromosomes broken at centromere regions do adhere to free ends, and vice versa, so that submedian chromosomes become terminal, or the reverse. This would seem to be the first evidence that ends resemble centromeres in some properties, a postulate for which this paper has produced still further evidence.

15. Summary

The meiosis of the male robber fly, *Habropogon appendiculatus* Schiner is described. It is similar to that of male *Drosophila*, but in addition the prophase stages are visible. There is an *X Y* sex mechanism, these chromosomes being over-condensed throughout prophase, and understained at metaphase.

Prophase is characterized by the presence of non-homologous associations between sex chromosomes and the ends and centromere regions of the closely paired autosomes. In these associations at early pachytene 60 % of the ends and 40 % of the centromeres of the autosomes take part. The sex chromosomes are involved about three times out of ten, and their low frequency of association is due to the presence of nucleolar material. The centromeres become progressively liberated during prophase, and very little centromere association persists to diplotene, and none beyond this. The end associations persist to diakinesis, and break up during this stage.

The cause of the non-homologous associations is discussed, and they are compared with similar associations in *Corixa*, and in polarized nuclei,

and with the chromocentre in salivary gland nuclei, and the method of comparative inference is used to show that these associations are not due to nucleolar material, and that it is unlikely that they are mainly due to heterochromatin. Evidence is adduced that their most probable cause is mutual attraction between ends and centromeres, and that the additional factor of centrosome attraction will account for the difference between the associations in polarized nuclei and those in *Habropogon*.

Heterochromatin probably plays a subsidiary part in some of these associations, as there is conclusive evidence that it can cause non-homologous association in salivary gland nuclei and in grasses.

The fact that the sex chromosomes are always closely associated with the nucleolus is attributed to the presence of nucleolar organisers within them, and the fact that they show no nucleolar constrictions to the lesser repulsion of the heterochromatin of which they are composed.

There is evidence that precocious condensation at prophase is correlated with proximity to the nuclear membrane, and it is suggested that this is because the synthesis of nucleotides, precursors of nucleic acids, occurs at the membrane.

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EXPLANATION OF PLATE 14

All photographs are of the first meiotic division; magnification × *c*. 3000

Fig. 1. Early prophase, stage C. Portion of cell illustrated in Text-fig. 6.

Fig. 2. Early prophase, stage C. Portion of cell illustrated in Text-fig. 7, showing an association between a centromere and two ends, without visible heterochromatin.

Fig. 3. Early prophase, stage C. Association of two autosome ends with the sex chromosomes, which are embedded in one side of the nucleolus.

Fig. 4. Middle prophase, stage D. Cell illustrated in Text-fig. 8. Association of centromere and two ends.

Fig. 5. Diakinesis, stage F. Cell illustrated in Text-fig. 15. Association of four ends.

Fig. 6. Metaphase. Cell illustrated in Text-fig. 19. The sex chromosome pair is situated at 6 o'clock.

Fig. 7. Metaphase. Cell illustrated in Text-fig. 20. Side view showing the close parallel secondary pairing of the autosomes.

Fig. 8. Anaphase. Cut cell, as in Text-fig. 22. Five autosomes and the undercondensed and understained sex chromosome.